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Too soon to build laser weapons systems

The belief that science fiction must inexorably be transmuted into fact appears partly to account for the wave of interest in laser weapons which has recently been sweeping the United States. Ever since Darth Varda (the villain in *Star Wars*) showed how easily opponents could be evaporated with a flick of a ray gun, it has been a question only of time before the Pentagon and its counterparts elsewhere should be discovered to be working on weapons systems based on lasers. In reality, the Pentagon has been spending about \$200 million a year for the past few years on the technology of high-energy lasers, and development may accelerate (see page 111). Some kind of laser system is likely to be one of the first military payloads to be carried aloft by the shuttle system. And there is an expectation (not yet falsified) that the new president will be more willing to put money into novel weapons systems, even harebrained devices, than his immediate predecessors. It is no wonder that laser weapons seem like weapons systems whose time has come.

A measure of scepticism is therefore in order, and has fortunately been provided in the past few weeks by a document (*High energy laser weapons — a technical assessment*) by M. Callaham and K. Tsipis of the Massachusetts Institute of Technology. The document is no more than a review of how laser weapons might be used and of the problems that will have to be solved before practical weapons systems could be put to work. Would-be Darth Vardas may regard it as a promise of goodies round the corner. More sober reading of the text suggests that even if the formidable technical problems which are all too apparent can be solved, there will persist serious physical limitations of what can be accomplished by laser weapons. In other words, there seems no immediate reason why anybody should fear that the already fragile framework of international relations is about to be transformed by yet another new technology.

The uses suggested for the non-existent laser weapons would gladden any Darth Varda's heart (if he, she or it has one). Offensive uses include the delivery of large amounts of energy on the heads of troops on battlefields and the destruction of other people's Earth satellites. But, on paper, laser weapons could also be used defensively for intercepting hostile missiles or at least aircraft. Laser systems can in principle be based on the ground or carried aloft, either in aircraft or satellites (and the United States has at present at least one Boeing 747 fitted out with equipment designed to study the propagation of laser beams in the upper atmosphere). In all cases, the objective is to exploit the coherence and potentially large energy intensity of a beam of laser radiation to damage objects or people. On paper, at least, directing a laser beam at chosen targets should be relatively easy, given that photons have no inertial mass. Perhaps fortunately, there are snags.

One is a snag well educated schoolboys will be aware of. Although it is customary and usually fair to suppose that a beam of laser radiation is a parallel pencil beam, Huygens's principle is not suspended for the benefit of military planners. The best-ordered laser beams spread out to some degree, and the effects are far from insignificant when people talk (as some do) of using lasers based in geosynchronous orbits for damaging objects at ranges of several tens of thousands of kilometres. In most of the applications dreamed up so far, damage is determined by the maximum intensity of the beam. At a range of 1,000 kilometres, the intensity of the infra-red beam from a carbon dioxide laser with an initial aperture of a metre would have declined by a factor

of 400 even in transmission through a vacuum. Propagation through the atmosphere entails more rapid degradation as a result of Rayleigh scattering by molecules, by turbulence and by absorption and scattering by water droplets or particles of dust. Operations below the stratosphere will be impeded by haze and clouds (although it is entirely feasible that laser beams could be made powerful enough to burn through some meteorological obstacles). But even in a clear atmosphere, the beams of high-powered lasers are degraded for reasons rarely encountered in the laboratory. Inevitably, there is a temperature gradient and thus a gradient of refractive index across an intense laser beam and the result is, predictably, that the beam is further broadened. The chances are that a good part of the Pentagon's expenditure on military laser systems is spent on the study of this phenomenon of "blooming". The obvious remedy is to work with ever shorter pulses of radiation, but there are limits to what can be done — if the power transmitted is to remain unchanged, there is a risk that increasing field strengths will turn the atmosphere into a plasma and thus render it opaque, or generate out of the surface layers of the target a similarly protective plasma skin.

Other physical obstacles to the development of laser weapons, problems of energy supply not least among them, promise to be just as daunting. Directors of science fiction films do not fully appreciate that lasers, like other sources of energy, themselves consume power. Even supposing ideal efficiency within a laser itself, which supposes that problems of heat dissipation within the optical system are somehow solved, the quantities of energy that must be beamed about the sky in the pursuit of military targets are certain to be huge. If, for example, the objective is to deliver something like 100 joules of energy to each square centimetre of the surface of a target at a range of 1,000 kilometres, the output of a laser would have to be some 400 megajoules, allowing for the spreading of the beam from a one-metre mirror. On paper, a few kilogrammes of hydrogen fuel (and eight times as much oxygen for combustion) might be sufficient for a single attack of such a kind. Such a rate of energy consumption might be tolerable for a ground-based weapon but would be entirely out of court, at least with present technology, for a system based in some orbit around the Earth. It is therefore not surprising that the calculations that Callaham and Tsipis have carried out, admittedly back of an envelope stuff, suggest that laser weapons systems might have masses of several thousands of tons. No doubt, by ringing the changes on pulse length, mirror diameter and the like, the people at the Pentagon would be able to arrive at better compromises, but there seems no doubt that laser weapons will be huge constructions, a little like particle accelerators in the complexity of their power supply.

Such obstacles are unavoidable. Others may be more susceptible to research and development but remain, as things are, quite overwhelming. One curious aspect of the recent excitement about laser weapons is the continuing uncertainty about the best way of using a laser beam to destroy a target. The simple notion that its surface might be melted or even vapourized entails that large quantities of energy should be delivered to the target. The mechanical shock caused by a pulsed laser beam may be a more economical way of causing damage, but is less predictable. This is clearly one area in which the military have ambitions for research and development. The technology of picking out targets at which to aim is another. Laser beams themselves may have no inertia, but the mirrors which fashion them are material objects that must be pointed in a direction



which is likely to intercept the intended target. Given the kinds of targets the planners have in mind, however, the computational and mechanical tasks that they must solve are formidable.

This is why the issue that will face the new Administration in Washington, and its counterparts elsewhere, is whether the game is worth the candle. For whatever virtues laser weapons may eventually be shown to have, there is no doubt that they will be uncommonly susceptible to relatively simple countermeasures. A simple false skin on every warhead would do the trick, while laser weapons would themselves be put off their stride by much less powerful hostile lasers. These are the reasons why even hawks in Washington are now asking that Mr Reagan should not decide to spend even larger sums of money than at present on the

development of laser weapons. For the time being at least, there are better ways in which the Pentagon could spend large sums of money. The time for going all out for laser weapons is not now but later, perhaps when somebody has a clear idea for a laser weapon system that could perform a tangible if limited role in the foreseeable future. Mr Reagan's opponents on laser weapons cannot, however, have it both ways. If the technical problems are as formidable as they say, and the opportunities as negligible, there is little danger that wasting money in this way would further disturb arms control negotiations with the Soviet Union. If there were a prospect that, say, a workable anti-ballistic missile system based on a laser might be developed within a decade, it would be a different matter. On present form, that danger is remote.

What if Britain wants not to change?

The Centre for Technical Change has made a brave start on a daunting problem — the diagnosis and, with luck, the cure of Britain's chronic problem of turning research and development into prosperity (see page 114). Sir Bruce Williams, the newly appointed director of the centre, is a prize catch. His appointment should go a long way to still some of the muttering about the centre's chances of success. Even so the centre's wellwishers will keep their fingers crossed. British institutions, but especially the civil service, habitually resist gratuitous advice, which is why organizations like the Brookings Institution have not flourished in the United Kingdom. The most obdurate of the problems which the new centre will have to solve is that of making people listen to what it has to say. To do this, it will need guile but also an imaginative, even a radical, agenda. Where should it begin?

There is no shortage of conventional accounts of what is wrong with the British economy, and with the linkage between the economy and technology. For the past thirty years, public support for research and development has been generous, even profligate, but only in retrospect has it dawned on people that much of this effort has been misplaced. Defence research, especially in aerospace and electronics, has taken the largest share of public funds. Throughout this period it has been assumed, in the face of experience and against reasonable expectations, that work carried out in secret in government laboratories would somehow filter through to revivify the civil economy. In retrospect, nobody is surprised that this has not happened, that the British aircraft industry has an annual turnover smaller than that of Boeing or that the British government is having to spend public money on last-minute attempts to foster the design and manufacture of microprocessors. Yet the huge defence laboratories remain more or less intact, ageing slowly as their staffs approach retirement.

During the past thirty years, British governments have inconsistently tried to remedy this state of affairs. They have encouraged the production of graduates in science and technology (1955–65) in the belief that such people would make a market for their services. They have encouraged the creation of large technical companies (1964–70) in the vain hope that the conglomerates would seize the opportunities which technology presents. They have wasted money and, worse, people's talents on glamorous projects with no economic benefit such as the Concorde project (inherently uneconomic) and nuclear power (where even successful innovations have been pigeonholed until recently). Throughout this period, governments have sought without conspicuous success ways of encouraging technical innovation in the general run of British industry, finding only that the small and medium-sized firms best placed to profit from radical innovation are the least able to raise the necessary capital.

This depressing record shows that there is no point in hoping that research and development, or skilled manpower, can by themselves improve the exploitation of innovation by British industry. Indeed, the chances that such an old-fashioned recipe would work are less than they have ever been. Industry, especially small industry, does not lightly think of spending extra on

research and development at times like these. (The exceptions are those companies, many of them in the chemical industry, which have learned in the past thirty years that research and development is not merely profitable but indispensable.) Moreover, there is little hope that the new centre will be able to produce such a more penetrating analysis than other analysts of the ills of the British economic system that the scales will fall from people's eyes, expectations of prosperity will be reduced to some amount commensurate with the Gross Domestic Product and the ills of inflation and deflation will be cured less painfully than by monetarism.

So where is the Centre for Technical Change to look for an agenda if these obvious fields of enquiry are likely to be ineffectual? Fortunately, not every hopeful door is shut. There are two particular fields in which the centre could do sterling service. First is the British education system, now most of all distinguished by its eccentric and inexplicable differences from other educational systems in other industrialized states. British first degree courses are shorter than elsewhere, but those who embark on them have to demonstrate in advance that they have already covered much of the ground. The result is that too many narrowly educated people, mock-specialists before their time, are turned out into a world in which their teachers are constantly declaiming the need for continuing education to meet changing needs. Throughout the educational system, the pursuit of academic excellence is properly regarded as a virtue. Improperly, the use of the educational system for the pursuit of something else, say wealth or even happiness, is regarded as a kind of sin. Occasionally, out of subliminal recognition that there may be an inconsistency somewhere, people like Sir Monty Finniston are persuaded to preside over inquiries into the parlous condition of this or that profession (engineering in his case). Perhaps they are lucky, since their inquiries are always so confined that governments usually decide not to act on their recommendations or (as in Sir Monty's case) to act halfheartedly. For a nation as preoccupied as the British with education, it is increasingly ridiculous that so much of what is said should make so little sense.

The second field in which the Centre for Technical Change should take an interest is closely related. British civility is, of course, renowned, and is probably also a product of the educational system. People do not openly seek to embarrass one another. The result is that casual acquaintances need not fear that they will have to disclose their salaries at dinner parties, which makes for congeniality. Unfortunately, the same principle applies in the real world. People in industrial research make their reputations by publishing in academic journals; occasionally they become (unpaid) visiting professors at a neighbouring university. Salesmen sell first to their sales managers, and afterwards to their customers. Rarely is it allowed that the pursuit of pound notes can be a seemly occupation — or that the consequences of success can ever be worthwhile. The problem for the centre, in tackling such a subject, is twofold. Is it capable of objective analysis and presentation? And would such an analysis, however rigorous, merely persuade the genteel British that things are better as they are?

Reagan assailed by laser weapon lobbies

Hawks say go: doves argue caution

Washington

An early confrontation seems to be brewing between the Administration of incoming president Mr Ronald Reagan and members of the scientific community concerned with international arms control over plans to develop laser weapons for use in space.

Key congressional Republicans are said to have been told by members of Mr Reagan's transition team for the Defense Department that he wants to increase research and development on the use of lasers to destroy incoming ballistic missiles. One report claims that an early launch of the National Aeronautics and Space Administration's space shuttle will test an aiming device for a space-based laser, a project code-named Talon Gold.

But these rumoured plans are coming under strong attack from scientists who claim that laser weapons systems would not only be extremely costly and of dubious technical value, but that an accelerated research programme could upset the chances of negotiating an agreement over how such weapons should be controlled.

Speaking at last week's meeting of the American Association for the Advancement of Science (AAAS) in Toronto, for example, Dr Richard Garwin, chief scientist at IBM's Thomas Watson Research Center, claimed that excessive emphasis on directed-energy space weapons diverts funds and talent from more serious and more effective threats.

Mr Reagan's quoted views are compatible with a report from the science and technology subcommittee of the Senate Commerce Committee, which says that if rumours of an impending commitment by the Soviet Union to develop laser weapons are to be taken seriously, then there should be a significant increase in support for the Defense Department's research programme in the area.

The Senate committee admits that there is an "unsettling" diversity of opinion within the US scientific community about the technical and economic feasibility of high-energy lasers in a wide variety of potential applications, including defence, but it points out that the present Department of Defense budget allocations for high-energy laser research are not sufficient to achieve operational weapons as early as would otherwise seem possible. An increase in funds and commitment by the department would, it says, "significantly accelerate the achievement

of directed energy weapons systems", a view apparently shared by leading members of Mr Reagan's defence transition team.

The Senate report provides some insight into the controversy about US efforts in laser weapons research that has simmered within the Pentagon for several years. The controversy surfaced in 1979 with reports in *Aviation Week* that the Soviet Union had begun to build a large high-energy laser at Sary-Shagan, a weapons testing area near the Chinese border, and that this appeared to be the first step towards a full-blown laser weapons system able to destroy both satellites and incoming missiles.

Defence officials have been divided on how to respond. Some — in particular, Dr William Perry, Under-Secretary of Defense for Research and Technology — have played down the potential threat, arguing that the gap between theory and practice was so large that the Soviet Union was unlikely to have a weapons system in operation until the 1990s, and rejecting claims that the Soviet Union had a significant strategic advantage in this area.

Others in the Pentagon have been dissatisfied with this reaction, and have been pushing for an aggressive laser weapons research programme, demanding substantial increases over the current \$215 million a year being devoted to the high-energy laser research, primarily in the Air Force and by the Defense Advanced Projects Agency.

The dominant thinking within the Carter Administration has been that the main emphasis should remain on research. The report of the Senate committee — apparently now in agreement with Mr

Reagan's advisers — is that research alone is not enough, and that "a balance between technology development and weapons system development" is needed.

The committee suggests, for example, that a special office be created within the Department of Defense to manage and direct the overall high-energy laser programme of the department, rather than distributing its research and development efforts across the different services. And it endorsed a bill introduced by Senator Howell Heflin of Alabama to create a National Laser Institute to coordinate all laser research and development.

Fears that such enthusiasm may be allowed to flourish relatively unbridled under the new administration are prompting the members of the arms control community to argue strongly that the national security implications of even an accelerated research programme should be carefully considered.

In a recent report from the department of physics at the Massachusetts Institute of Technology, Kosta Tsipis and Michael Callahan point out that, over and above the severe technical and economic hurdles facing the development of a full laser weapons system, even a research programme could be upsetting to strategic arms limitation talks, since it would be difficult to tell whether the research was for defensive or offensive purposes.

In his paper to AAAS, Dr Garwin argued in a similar vein that space-based lasers would be highly vulnerable to attack by anti-satellite rocket vehicles, which could be developed with a fraction of the resources required to install the space weapons.

David Dickson

European science portfolios still for grabs

Brussels

The division of portfolios among the new members of the European Commission last week has left scientific research in the dark. The next few days should give the *cabinets* of the principal commissioners, Etienne Davignon and Ivor Richard, the new British commissioner, a chance to sort out their division of responsibilities.

So far it seems that Davignon, a Belgian viscount, will be taking over responsibility for all energy and industrially related matters, while Ivor Richard will take over the rest of research, science and education. This will involve some reshuffling of the bureaucracy, but nobody is prepared to predict what effect these changes at the top will have on policy.

Davignon, now entering his second four-year stint as commissioner in charge of industrial affairs, has become one of the most powerful figures in Brussels through his handling of the steel crisis. He takes over responsibility for energy from the now

departed German, Guido Brunner, and has already made it clear that he aims at a tough energy-orientated policy to revive European industry.

His chief interest in research is likely to be the development of energy-efficient technology, alternative energy sources and research aligned with industrial innovation. A notable advocate of Community programmes in telematics (the marriage of chip technology and telecommunications) and bioengineering, it is doubtful whether, with the broadening of his responsibilities, he will have much time to devote to the poorer aspects of Community research programmes.

This leaves Ivor Richard in charge of the joint research centres, education and training, which will tie in neatly with his responsibility for employment and social affairs. Another new commissioner, Karl-Heinz Narjes from the right-wing German CDU party, has been given undefined portfolios for industrial innovation, nuclear safety and environment and consumer pro-

tection. Narjes, like Davignon a lawyer by training, is regarded as being for nuclear power and, with little in his past to suggest that he will be particularly radical in his approach to environmental issues, his coming is regarded with some apprehension.

Ivor Richard's past experience in the Labour government includes the post of parliamentary under-secretary of state for defence in 1969–70 and deputy spokesman on foreign affairs between 1972 and 1974. He was the British ambassador to the United Nations from 1974 to 1979. Previously he had been a Council of Europe delegate and, during the same period, 1965–69, had been with the West European Union.

The new commissioners as a whole are largely right-wing men with legal backgrounds. They include nobody with previous experience in scientific affairs. It may be an advantage, however, that Richard, without any other major responsibilities, will be in a position to give his full attention to promoting the interests of his directorates-general. Davignon is certainly keen to increase the Community's influence on the industrial research programmes of the member states. It will clearly be some time before the new Commission settles down and begins to exert an appreciable influence on Community policy; undoubtedly the immediate problems associated with the budget and the form of the Common Agricultural Policy will take precedence for the time being.

Jasper Becker

Nucleotide sequences

Too many banks?

The US National Institutes of Health (NIH) and the European Molecular Biology Organization (EMBO) seem well on the way to founding a computerized bank of nucleotide sequences, but would-be competitors have not yet withdrawn from the field. The joint NIH/EMBO plan springs from meetings in Bethesda (Maryland) and Heidelberg last year, both of which acknowledge that nucleotide sequencing techniques now in use are likely to generate more information than can be assimilated informally.

For the time being, nucleotide sequences are being collected at EMBO headquarters in Heidelberg by Mr Greg Hamm. Other centres interested in the collection of nucleotide sequences include the George Washington University, Washington DC, where Dr Margaret Dayhoff plans to broaden the scope of her collection of amino acid sequences; the Los Alamos Scientific Laboratory; and Professor Richard Grantham's CNRS *Laboratoire de Biometrie* at Lyon.

The work now being carried out at Heidelberg is regarded as a necessary preparation for an eventual long-term decision by EMBO and NIH. It is by no

means certain that the two institutions will continue to collaborate, although such an arrangement is seen as a potentially valuable precedent. In the meantime, the Heidelberg collection is being compiled and made available informally.

The outstanding technical problems are more concerned with molecular biology than with computer technology. Storing, retrieving and comparing nucleotide sequences stored in computer language can be tackled with existing programs, but decisions have yet to be made about the criteria by which sequences should be considered authentic, while the problems of merging incomplete sequences with each other, and of accommodating and authenticating variations, will entail something like a critical evaluation of data in molecular biology.

The NIH/EMBO plan assumes that nucleotide sequences will eventually be made available to all suitably qualified scientists, but also supposes that the effective management of a sequence repository will require the continuing presence of a small group concerned with the analysis of sequence data.

Professor Richard Grantham's plans are similar. His bank is said to contain all published sequences of more than 150 nucleotides, but tRNA and 5S rRNA sequences are not included because of their variability. At the end of September, the Lyon bank included 200 mRNA sequences together with 10 complete genome sequences and 100 untranslated sequences. A description of the bank and a list of its contents can be had free of charge from Université Lyon 1, 69622 Villeurbanne Cédex, France.

It is hoped that the future of the NIH/EMBO project will be decided early this year, after consultations with other interested parties. One of the potentially contentious issues is that of whether bodies such as EMBO and NIH should centralize an activity recognized to be essential but traditionally left to individual research workers relying on grants from similar bodies.

Polish pollution

Smelter shuts down

Poland's new Ecological Society has won a major triumph in its battle to halt pollution in Krakow — a ministerial promise that the Skawina aluminium works, one of Poland's two major producers of the metal, will be closed down entirely. The campaign against the Skawina works has been going on since the society was founded last September.

The post-war industrialization of Krakow, beginning with the building of the giant Lenin steel mills at Nowa Huta, has brought major pollution problems. By the end of the 1970s, Krakow, which lies in a hollow with little natural circulation of air, annually received an estimated 95,000

tonnes of dust and 70,000 tonnes of sulphur dioxide a year from Nowa Huta to the east, and 33,000 tonnes of dust and 33,000 tonnes of sulphur dioxide from the Skawina complex (a coal-fired power station and aluminium plant) to the south-west. Ecologists working on the former Royal Forest of Niepolomice some 20 km from Krakow had to recommend measures such as aerial spraying with lime to neutralize the acid rain.

Outside the technical journals and reports, little was published on the problem, although the local population could see for themselves the outpourings of smoke (new electrostatic precipitators were constantly being promised) and there were increasing fears of the health hazards of locally grown vegetables. The first real public hint that there was an even greater gas hazard than that of sulphur dioxide came in the autumn of 1979, with the revelation that gold art treasures in the Wawel Castle museum were becoming corroded.

A year later, however, the new Ecological Society, whose membership includes representatives of the "interested public" as well as experts, was demanding a "truthful report" on Krakow's monuments and environment including public health issues. The society's memorandum, published in the Krakow newspaper *Gazeta Poludniowa* of 20 November, demanded in particular that all processes emitting fluorine and hydrogen fluoride must be shut down until this hazard could be eliminated. In fact, both the Skawina works (the main source of fluorine pollution) and the Nowa Huta steel mill were already scheduled for modernization — at costs of 120 million zloty (£2 million) and 7,500 million zloty (£125 million) respectively. Preliminary work on these projects, it was promised, would begin during 1981.

But the debate continued and was taken up by the national television network. By the end of December, the Minister of Metallurgy, Zbigniew Szalajda, had agreed to a partial cut-back in production, and ordered 35 obsolete electrolysis tubs to be taken out of service. The Mayor of Krakow, Josef Gajewicz, increased the number to 160, cutting hydrogen fluoride emission by 60 per cent and aluminium production by almost half. On 5 January this year, the minister told the press that Mayor Gajewicz's action would produce a "very difficult situation" for manufacturers requiring aluminium. Ways would have to be found to step up production from Poland's other smelting plant at Konin. The ministry's plans for the future of the Skawina plant, he said, would be outlined shortly. Two days later, after a meeting with community representatives and journalists in Krakow, the minister announced that, "taking into account all the circumstances, the demands of the community and the economic aspects", the plant would be closed and the project "totally abandoned".

Vera Rich

Human experimentation

Indian rules

Lucknow

To help Indian investigators plan their clinical studies the Indian Council of Medical Research (ICMR) has formulated guidelines for conducting medical research on human subjects.

The guidelines have been prepared by the ICMR Central Ethical Committee whose chairman is a legal expert and whose members include the Drugs Controller of India. Briefly, they seek to ensure that the rights and welfare of subjects participating in a trial are protected, that the benefits accruing from the research outweigh the risks to the individual, that the "informed consent" of the subject is obtained before the start of the trial, and that the investigator has the competence and experience to conduct the trial.

ICMR has forwarded the guidelines to the Indian government and hopes that they will be followed by all institutions engaged in clinical research in the country. Institutions under the aegis of ICMR have already been instructed to follow the guidelines. It is suggested that, to implement the new code, each institution conducting clinical research should form its own committee, which would consist of five to seven experienced clinicians and non-medical persons able to give advice on legal and ethical matters. These committees would then evaluate each proposal for research on human subjects by following a nine-point code. ICMR recommends that, if the ethical committees are to be effective, they should be independent bodies.

Recognizing that it may take some time for each institution to form its own committee, ICMR has offered to provide the services of an ethical committee to evaluate projects during an interim period of one year. This new role for ICMR will enable Indian institutions which do not have ethical committees to apply for funds from international agencies such as the World Health Organization, which will not consider a proposal for research support unless approved by an ethical committee.

The ICMR guidelines stress the importance of obtaining the informed consent of each subject. Although the details for obtaining consent are left to individual committees to work out, ICMR insists that the subject or his guardian should be given as much information as possible about the drug to be studied, including its side effects, and that it should be made clear to him that he is free to refuse to take part in the trial.

Indian law demands that the permission of the Drugs Controller of India is obtained before a clinical trial is performed on a new drug. ICMR proposes to hold meetings with other organizations involved in clinical research, to formulate ethical safeguards that will prove generally acceptable.

Zaka Imam

Vitamin deficiencies

Rickets at bay

Vitamin D-deficiency rickets appears to be on the decline among Britain's Asian community. The Department of Health and Social Security last week reported* a fall in the incidence of rickets among Asian children in the past ten years, but says that the disease remains a problem. Seeking to eradicate the disease, a department working party recommends vitamin D supplements for children at risk rather than the fortification of specific foods, milk or chapatti flour.

Once known as the "English disease", rickets was common in Victorian England with as many as a half of the children in poor areas in Leeds said to be suffering from it at the end of the nineteenth century. In the period 1926-42, the incidence was still high, with some 2-8 per cent of children in British cities with the condition. During the Second World War, however, rickets was almost eradicated by a programme (begun in 1943) in which all children received vitamin D in the form of cod liver oil supplements. The hope now is that the same approach could work among Asian children now at risk.

Rickets and its adult equivalent, osteomalacia, were first documented as a serious problem in the Asian community in Glasgow in the early 1960s, and since then has been observed in most of the larger Asian communities in the United Kingdom. Neither disease is notifiable, however, and data collection is not easy.

The recent decline of incidence is suggested by figures from the Hospital In-Patients Enquiry Programme and from a survey involving 130 general practitioners in areas with large immigrant populations. In 1977, in the Midlands and north of England, ten Asian children in 1,000 under the age of 16 had rickets, compared with only four per 1,000 in London. The risk factors are said to be lack of sunlight, strict vegetarian diet, poor rural background and a family history of rickets or osteomalacia. As both bone diseases are common in the Indian subcontinent, the report suggests that immigrants may bring it with them.

Thus, the department says, it should not be difficult to identify those at risk, which is why its report recommends vitamin D supplements backed up by health education. The decision against food fortification has been recommended by the department's Committee on Medical Aspects of Food Policy, which has studied the problem for four years, but will not satisfy all clinicians responsible for treating rickets in the Asian community.

The case for the more radical programme of fortifying specific target

**Rickets and osteomalacia.* Report of the Working Party on fortification of food with vitamin D, Committee on Medical Aspects of Food Policy, Department of Health and Social Security. HMSO, £3.90.

foods has fallen because legislation would be required before vitamin D could be added to specific foods such as milk or flour, and because the food policy committee has argued that there are sound medical reasons for not taking such a course. Thus there might then be excess vitamin consumption and, as a consequence, the risk of hypercalcaemia in children and possibly of cardiovascular complaints in adults.

In retrospect, the hypercalcaemia observed in young infants in Britain in the 1950s was almost certainly due to vitamin D overdosing. The department's report says that if milk were to be fortified with vitamin D, some children could well receive too much, especially if concerned parents also choose to provide vitamin supplements.

Technical problems complicate the fortification of chapatti flour — the target food best suited to the needs of the Asian community in Britain. Although vitamin D is stable in the flour, up to 50 per cent can be destroyed in cooking. Choosing the correct amount to add to flour is therefore difficult and the concentration of vitamin D needed to prevent rickets in young children might well prove a health risk to teenagers and adults.

Alastair Hay

Research development

Soliciting custom

The National Research Development Corporation (NRDC) is trying to improve its image. To that end it held a meeting in Manchester last week to spread its message among leading academics, industrialists and financiers in the north-west. The meeting was the second of its kind, the first having been held in Cambridge about a year ago, and it is unlikely to be the last.

NRDC, which exists to help exploit technological innovation in industry, clearly believes that not enough demands are being made on its funds and services and that more universities, and especially industries, should be coming to it with their ideas. It supports fewer projects in relation to the number of higher education and research establishments and industries in the north-west than in any other region — hence the choice of last week's meeting venue.

To judge from their criticisms, however, few of those involved in the business of technological innovation regard NRDC as a particularly helpful body. Many academics, who are obliged to offer NRDC first refusal of inventions resulting from publicly funded research, say that it is too bureaucratic, especially in securing legal protection for non-patentable inventions such as computer software. It is also criticized for not being sufficiently aggressive in marketing inventions, and for taking both too few and too many risks. Its high failure rate, as much as 80 per cent in some fields, can be taken either as a healthy

sign of its willingness to take risks or as a reluctance to match its patent portfolio to its financial resources.

Most of the inventions communicated to NRDC which later result in commercial success come from government research establishments, universities and industry. In the year up to March 1980, 1,786 inventions were communicated to NRDC, 68 patents were assigned to it, 56 licence agreements were completed and 157 development projects were set up. For those working outside industry, the corporation offers to patent ideas, put up funds for further development to the point of commercial exploitation and then licence the inventions with industry.

NRDC's aims seem laudable enough, but academics in particular feel that the practice does not match the theory. Those who have been able to approach industry directly with their inventions often report greater success, largely because they are able to negotiate relatively informal deals which do away with the legal wrangling often associated with NRDC negotiations. Universities have also become increasingly aware of their science and technology departments as potential revenue earners.

For industry, however, NRDC's services may seem more attractive and it was at industry that last week's conference was primarily aimed. If academics criticize NRDC for not driving hard enough to exploit ideas, the NRDC may equally accuse academics of lacking motivation. Appealing to industry itself to come forward with its own inventions might be more productive.

Judy Redfearn

British technology

New direction

The Centre for Technical Change, the technological think-tank set up in Britain last summer, has brought off a considerable coup by the appointment of Sir Bruce Williams (aged 62), Vice-Chancellor of the University of Sydney, as its first director. The appointment, announced last week, will take effect full-time when Sir Bruce bows out of his post at Sydney at the end of June, two months early.

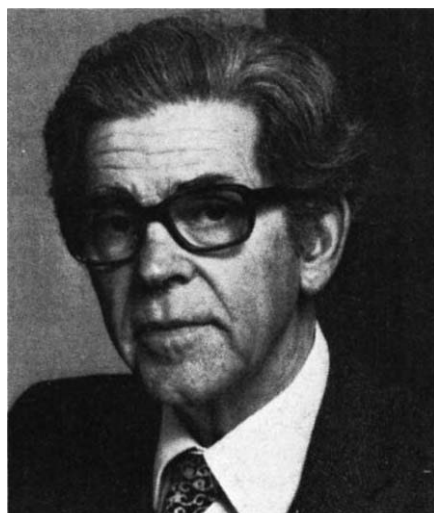
The centre was set up with a grant of £1.5 million from the Leverhulme Trust and with the promise of further substantial funds (up to a total of £2.25 million over five years) from the Science and Social Science Research Councils. Its origins lie in the conviction of its sponsors that pure reason may help to explain the poor industrial performance of the United Kingdom in recent decades, especially in the exploitation of technical innovation.

One of the ironies of Sir Bruce Williams's appointment is that it is almost exactly 25 years since the appearance of a report on the same set of problems produced under the title *Science and Industry* by Williams and Dr (now also Sir) Charles Carter, recently retired Vice-

Chancellor of the University of Lancaster and now director of research at the Policy Studies Institute, London. That document raised the question of why Britain, one of the principal spenders on research and development, should apparently derive so little benefit as a consequence.

Detailed planning of the centre's work is still incomplete. It has however been decided that the centre should be based in London, not (as some had hoped) in a university town.

It was also announced last week that the deputy director of the centre will be Dr A. J. Kennedy, research director of Delta Metal Company Limited. Dr Kennedy, who is 59,



Williams — back to a sinking ship?

has a background in academic life (Cranfield) and in industrial research. The centre is managed by a council whose chairman is Lord (formerly Sir Michael) Swann, now Provost of Oriel College, Oxford. Its future is for the time being assured only for five years, but it is hoped that it will be able to recruit extra funds during that period and also to persuade its sponsors that it deserves a longer life.

Latin America

Freedom lost

Washington

As one of a set of new initiatives designed to help persecuted scientists in Latin America, officials of the American Association for the Advancement of Science (AAAS) intend to monitor loans provided by international development banks to research and training institutions in the subcontinent.

This action is a response to evidence presented to a workshop held during AAAS's annual meeting in Toronto last week that several loans may have been granted to Latin American institutions to compensate for the loss of scientists who have fled abroad.

There was particular concern over two loans — \$66 million lent to Argentina in 1979 by the Inter-American Development Bank (part of the World Bank) to train

scientific and technological research workers at nine regional institutes, and a \$32.5 million loan last autumn to the University of Uruguay.

According to participants at the meeting, one of the purposes of the Uruguayan loan is to employ foreign faculty and consultants on a temporary basis. These workers would help to rebuild science programmes, particularly in agricultural research, that had disintegrated following the recent wave of political repression, when many university teachers had been imprisoned or had left.

The workshop recommended that scientific organizations should encourage international lending institutions to consider human rights when granting loans to educational and scientific institutions. They stressed the need to establish beyond doubt that scientific and academic freedom would be preserved.

The workshop's recommendations were made in the light of evidence that the repression of scientists in particular — and educationists in general — is becoming a chronic problem in many Latin American countries.

Several participants admitted that the situation in some of the countries had improved slightly in the past few years. There are fewer reports of repression from Brazil and Chile than in the early 1970s, and even in Argentina — Buenos Aires physicist Dr José Westerkamp said things were no worse than in the past.

But Dr Westerkamp added that there was still widespread fear that the repression might come back again. And AAAS's Committee on Scientific Freedom and Responsibility, which sponsored last week's workshop, has turned its attention to countries such as El Salvador and Guatemala, citing evidence of "generalized violence" against university and other teachers.

The evidence presented of a general decline of academic and scientific freedom in Latin American countries, including the dismissal of many scientists from teaching and research posts, was sufficient to persuade the AAAS Council, meeting last week to call for "the exploration of new initiatives to protect scientists".

One of these initiatives is likely to be the setting up of a Latin America Regional Centre for Human Rights — possibly in Venezuela — to monitor the state of scientific and academic freedom in the subcontinent, since several participants stressed the need to involve indigenous scientific societies.

Meanwhile AAAS officials said that the association hoped to invite officials from the Inter-American Development Bank to a meeting of its scientific freedom committee, and pointed to the success of international conservation groups in slowing down one loan because of its potential environmental consequences as evidence that bank officials seemed willing to discuss such issues.

David Dickson

CORRESPONDENCE

Explosive reaction

SIR — Lovins' ¹ review "Nuclear weapons and power-reactor plutonium" lacks mention of prior rebuttals to his position^{2,3}. Moreover, in following the transition of this article with a widely circulated draft (personal communication, 20 March 1978), one senses a *post hoc* justification for his testimony at the Windscale nuclear-fuel reprocessing hearings.

The conclusions drawn by Lovins are unsupported by the analysis. He implies that "power reactors are not an implausible but are rather potentially a peculiarly convenient type of large-scale Pu production reactor". If power reactors were such practical sources, nuclear-weapons states would not be operating special-purpose military plutonium production reactors — they would be buying up reactor-grade plutonium for their weapons programmes, instead of storing it in pools. Reactor-grade plutonium is a dangerous substance, able to sustain a nuclear explosion. But Lovins fails to quantify its military value.

For governments that contemplate becoming nuclear-weapons states, the accessibility of plutonium is only one factor: others are the quality of material, the availability of facilities, and the possibility of nuclear-explosive testing. There are numerous disincentives too.

For "amateurs", there are hurdles not mentioned by Lovins. In general, plutonium generated in power reactors is not suited for military applications, and unauthorized access has been severely restricted by physical safeguards. His superficial treatment does not help our common goal of minimizing the propagation of nuclear weapons.

There are some specific technical objections: His introductory paragraphs are not justified by the subsequent analysis. He applies such terms as "unsuitable", "usable", "inferior", and "less than optimal", to power-reactor Pu. He generates a list of "assumptions" that represent selected extremes, later to render them "false". Although he says "We must know exactly what 'less than optimal' [Pu for bomb-making] means", he does not define it. Lovins eventually admits that there is "somewhat greater technical difficulty in using power-reactor Pu for effective military bombs."

His "conservative assumption" that arbitrarily high even-isotope content will not affect his arguments has been challenged, although Lovins does not mention the contradiction. On that assumption, Lovins proposes that "Pu discharged from power reactors will... have major military potential". His Table 2 does not reach high concentrations of the even-plutonium isotopes, thereby preventing the reader from making independent judgements on the efficacy of isotopic denaturing at higher even-isotope concentrations. Independent estimates of dramatic reductions in explosive yield that accompany isotopic dilution^{3,4} are not included in Table 2.

He categorizes his choice of parameters as "realistic", thereby dismissing the deleterious effects of other forms of denaturing. Lovins claims that my "analysis of weapons physics seems deeply flawed". That is the entire support he gives to the charge. Yet, his own "weapons physics" is sparse, lacking any equations, and sustained mainly with citations

from sources which I consider discreditable.

There are many misunderstandings, oversimplifications and omissions in Lovins' treatment of near-critical fissile masses: the difference between reactivity and excess reactivity; the role of synergistic effects; and his inability to distinguish between laboratory and field developments. I have identified and studied eight phenomena that can adversely affect the yield of an explosive configuration: critical mass dilution, metallurgical phase change, radial compression, subcritical multiplication, predetonation, generation time, reactivity limit and surface leakage. Lovins ignores most of them.

The interpretation that "the least favourable moment cannot get any less favourable" for an explosive yield is contradicted by Poisson statistics — a predetonation contour that Lovins notes but fails to apply. His repeated conception that the "worst case, minimum, 'fizzle' yield is still... 'militarily useful'", is inconsistent with probability theory.

Examination of the citations taken by Lovins from Wohlsetter and Gilinsky indicates the origin of his circular arguments regarding plutonium denaturing. Of the limited official information generally available, most current reviews depend heavily on R.W. Selden's unpublished conclusions, which are self-contradictory.

It is incorrect to state "categorically that bombs from reactor-grade or deliberately 'denatured' Pu are less effective, less powerful or less reliable than those made from weapons-grade Pu". On the other hand, using a term from Lovins, it is "disingenuous" to deny that those attributes may be applied to the average properties of denatured plutonium. I estimate that plutonium can be isotopically denatured as effectively as ²³⁵U or ²³³U at a given fissile fraction. All reactor fuels and reactor types pose comparable levels of difficulty regarding weapons application, given corresponding safeguards and fissile fractions. Weaknesses of one or the other should be identified and remedied. However, the relative reactor usability and supply of isotopically denatured plutonium is another matter.

The point that the "marginal time and money required to use civil reactors for military production are orders of magnitude less than those needed for dedicated military facilities," has an element of truth mixed in with carefree terminology. One must differentiate between base power reactors and small research reactors. Research reactors do indeed provide a hedge, a "civilian cover" for some weapons-grade plutonium production; however, power reactors are self-protected and inaccessible under an appropriate safeguards regime.

Institutional and technological safeguards can minimize diversion and proliferation. As our respective nations expand their arsenals of destructive weapons, such misplaced concern only adds to irresolution. One can transcend some of the dispute by noting that international safeguards and a ban on all testing of nuclear weapons are the most prominent and effective steps that should be taken.

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Role for ESF?

SIR — One aspect of its work that the European Science Foundation (ESF) could substantially expand (*Nature* 20 November 1980, pages 202, 204) is the sponsoring of scientific conferences and schools, especially in interdisciplinary areas.

The association of NATO Advanced Study Institutes and NATO Summer Schools with the NATO military pact is a matter of continuing shame to the academic community. Scientific organizations should be pressing the national governments to agree to the ESF taking over NATO's role in conference sponsorship and funding in Europe. The US side could presumably be accommodated via bilateral NSF-ESF arrangements.

Let's end the shameful military link with scholarship forthwith!

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Theories of cancer

SIR — In reviewing my book *The Politics of Cancer*¹, Peto² charged that one table (Table 6.4) selectively and deliberately omitted data that would otherwise have questioned the conclusion that low dietary doses of saccharin (0.01 per cent) are carcinogenic in rats. This statement is incorrectly based on comparison of Table 6.4 with data in an appendix to the report of the Office of Technology Assessment³. However, the caption of Table 6.4 clearly states that it is based on another table, prepared by Melvin Reuber for use in the congressional testimony on saccharin by the Health Research Group on 21 March 1977⁴. Peto persisted in publishing this serious allegation in spite of two warnings.

Peto also appears unfamiliar with the content of the Office of Technology Assessment's report, which he cites as the basis for his charges on saccharin. This report explicitly discusses Reuber's low-dose data and his conclusion that "the increased incidence of lymphosarcoma of the thorax in rats at the 0.01 per cent... are highly significant in the saccharin study"⁵. Reuber also emphasizes that the carcinogenic effects of saccharin in various studies were not always dose-related.

Contrary to Peto's impressions, inversions in dose-response data are not uncommon in both experimental and epidemiological carcinogenicity studies. Reasons for such inversions include competing risks, heterogeneity in tested populations, and statistical fluctuation, particularly when dose-response curves are shallow. Peto also fails to recognize that saccharin has produced tumours in experimental animals at low as well as at relatively high doses.

Contrary to Peto's impression that "it is not surprising that so many chemicals (such as saccharin) at such (high) doses can cause cancer in animals", there is an overwhelming consensus in the qualified, independent

scientific community that high-dose testing does not produce false positive results, and that this is necessary to reduce the insensitivity of carcinogenicity tests, reflecting the small number of animals tested compared with large human populations at presumptive risk⁶. It is also well recognized that carcinogenicity testing in excess of maximally tolerated doses (MTD) can produce false negatives due to competing toxicity.

Peto's charge of selective omission of the saccharin data is not his only misrepresentation. After his circulation of drafts of his review to various US experts in the autumn of 1979, Schneiderman, then Associate Director for Science Policy of the National Cancer Institute (NCI) and co-author of the government report on the importance of occupational carcinogens⁷, explained in a letter to Peto that breast cancer correlates as well with Gross National Product as fat, that occupation had been ignored in studies exclusively associating lung cancer with smoking, that there have been major recent increases in lung cancer among non-smokers and that there have been "big and frightening" recent increases in cancer incidence which cannot be accounted for by smoking or other lifestyle factors.

Similarly, Upton, then Director of NCI and a co-author of the report⁷, challenged the erroneous charge that a "4-5" multiplication factor was used to inflate the 1978 government estimates of cancer anticipated from occupational exposures. "... This simply is not true ..."

Repeatedly, Peto dismisses as personal views statements in *The Politics of Cancer* to which he takes exception, rather than recognizing that they are based on fully referenced primary sources and without attempting to challenge these sources directly. For instance, he disparages the conclusion "that the cure rates for major cancers have not been improving much over recent years" without noting that this reflects cited NCI data. Peto refers to the "claims that cancer costs the US economy over \$25 billion per year" without recognizing that such figures are derived from NCI sources. Similarly, Peto criticizes as "misleading or unbalanced" references to the term "medical-industrial complex" without attribution to its source, referenced and identified in the text (p.77), as the caption of an editorial in *The Lancet*⁸.

Peto charges that the book denigrates the value of short-term carcinogenicity tests, which he asserts is a "most inexplicable error of scientific judgement". While problems of such tests, particularly the limited associations between carcinogenicity and Ames test data for compounds from a wide range of structural classes^{9,10} are recognized, the book concludes (p.68) that there is a "range of useful applications" for these tests, particularly when incorporated into battery protocols.

Peto accepts that industries "delay or obstruct any hygienic measures which will cost money, ... (and) that the scientific literature is not immune from distortion by financial interests". Peto apparently also accepts the wide range of case studies in *The Politics of Cancer*, which document a common pattern of constraints, including manipulation, distortion and destruction, in health, safety and economic data generated or interpreted by industry and its consultants. Yet he seems willing to accept studies sponsored or endorsed by industry as authority for the nearly

exclusive lifestyle theory of cancer causation. Moreover, he is opposed to further regulatory controls on grounds of costs, professed "inactive conservatism", and because "for most toxic chemicals, we now have both qualitative and quantitative uncertainty about the health benefits of restriction". This seems a questionable basis for prudent public health policy.

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The article "Fallacies of lifestyle cancer theories" by S.S. Epstein and J.B. Swartz begins on page 127 of this issue.

Spanish science

SIR — The publication (*Nature* 23 October 1980, p.674) of the *Manifiesto* on the status of science in Spain issued by leading academics and scientists of universities and research institutes is a major event. I would like to make a few comments from the viewpoint of a scientist who has been working closely for over ten years with colleagues at Spanish universities.

This is a time of great expectations both nationally and internationally for Spanish science and engineering. Democratic changes and heightened national expectations have opened the door for a major strengthening of scientific, engineering and technological work. The largely dormant past in universities and the undeveloped governmental infrastructure for science are giving way to a healthy stirring throughout the whole system. A recent visit to Madrid confirms a vigorous self-examination of the structure of research and teaching with significant organizational changes proposed.

There is, of course, no unique set of circumstances that will guarantee motivation of faculty and students, but there generally appear to be three essential elements. Scientists must be assured of government respect for scientific work without political interference; there must be reasonable financial support and job opportunities for researchers and graduate students; and there must be adequate and respected links between government, universities and public and private research institutions. Only the first element is now firmly in place. Difficulty in providing the second element is not unique to Spain, but occurs in many industrial countries of moderate size where the number of research positions is limited. At the moment, for this reason, some of the best Spanish science and engineering graduates see their future abroad.

Academics might themselves develop research institutes within the universities directed to national needs such as high technology, engineering services, mitigation of earthquake and other natural hazards, and agricultural improvements. Although such efforts are exhausting and time-consuming, entrepreneurship to draw in industry, provincial governments and private funds is an essential part of scientific vigour. The *Manifiesto* appears to address largely the central government. No doubt, realistically at this stage, the major funding for research must come from Madrid, but, in the long run, a diversity of support would appear to be the most fruitful.

The third element should be easier to achieve since it does not in itself entail much expenditure. The need to examine critically scientific goals and infrastructure related to national needs has in other countries led to the establishment of something like a Royal Commission with independent powers to probe, report and recommend. Strengthening and linking government institutes undertaking research, similar to the highly effective scientific and industrial research organizations of Australia, New Zealand and elsewhere might be considered. There appears to be a need to integrate government scientific institutes more closely with universities, given the new social climate in Spain in which protection from debilitating political agitation is not required.

From my own experience, it is crucial for scientists and engineers in Spain to organize themselves systematically so that their professional societies and academies are representative and respected. The work of such associations can clearly demonstrate to the body politic (of all parties) that among the many competing interests in a modern state, science should have a high priority on both economic and cultural grounds. Rather than an emphasis on "rights", continual demonstration is needed of the way that scientific enquiry really maintains itself in a free society. Again, from my own experience, it is important to cultivate those members of the *Cortes* who are scientists, engineers, technologists and economists. If they are to accept the responsibility of speaking for the Spanish scientific community they must have the necessary contacts and realistic proposals that can be argued effectively.

Finally, as a sympathetic outsider, I am struck by the role that Spanish science and technology might play in the developing world. Much of Central and South America lies open, one would suppose, to the establishment of close ties with Spanish scientists and institutions. The value of common language and other heritages cannot be overemphasized. Although some cultural and scientific ties have been forged, there are exciting possibilities for an expansion of teaching, scientific exchanges, bilateral scientific and technological agreements.

Through such channels, the idealism and abilities of many Spanish science graduates could find a natural place that serves not only national self-interest but also the global responsibility of all scientists. Might we not look forward to a vigorous Spanish science that belongs not only to Europe but also to the Americas? Part of the answer lies in the force of the *Manifiesto* itself.

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NEWS AND VIEWS

Strife over visual cortical function

from D.M. MacKay

It is just over 20 years since Hubel and Wiesel¹ reported that cells in the striate cortex of cat show a preference for edges or bars of luminance-contrast at specific orientations. It seemed attractive to think of such cells as edge- or line-detectors, performing the first steps towards pattern recognition by analysing the retinal image into an array of oriented line-elements. When it was discovered that the cells also respond preferentially to gratings of specific spatial frequency-bands, however, a rival theory was advanced that they were 'really' frequency-filters analysing the image into its spatial Fourier components².

Complementarity of spatial and frequency analysis

But is this a genuine either-or? A simple cell can be thought of as a device for sampling the spatial distribution of luminance on the retina. As such it is subject to some limitations as first elucidated by Gabor³ in the context of communication engineering. He showed that if we want to represent a communication signal by a succession of elementary signals (samples) in the time domain, we have to accept a compromise

between the 'spread' of each sample in time and in frequency. The imprecisions of our representation in frequency (Δf) and time (Δt) are related by an 'uncertainty principle': $\Delta f \cdot \Delta t \geq \frac{1}{2}$ (ref. 4). For the product $\Delta f \cdot \Delta t$ to be a minimum, the elementary samples should not have sharp cut-offs in time or frequency. The best compromise is a sine- or cosine-wave whose amplitude is modulated by a gaussian function. It was later⁵ argued for analogous reasons that functions of space (such as optical images) would be most economically analysed in terms of elementary spatial functions with gaussian profiles. Four examples of such functions, adapted from Gabor³, are shown in Fig. 1a, b, with their spatial-frequency spectra in Fig. 1c. This means that any linear system with a spatial response profile of the kind illustrated in Fig. 1a, b is bound to show a preference for a restricted band of (spatial) frequencies. The narrower the spatial profile (Δx), the poorer will be the frequency selectivity.

As it happens, the receptive field profiles of simple cells (Fig. 2) are remarkably similar to the right-hand examples in Fig. 1a, b. Some are 'sine' in type (Fig. 2a); others 'cosine' (Fig. 2b). To the extent that they respond linearly, then, simple cells cannot help showing some selectivity also in the spatial-frequency domain⁶. In communication-engineering terms, their spatial 'spread' (Δx) is of the order of λ (Fig. 2). Their spatial-frequency spectrum is also near-gaussian, with a 'spread' of $\Delta f(x)$ given by the 'uncertainty relation': $\Delta x \cdot \Delta f(x) \geq \frac{1}{2}$. Taking Δx as approximately the wavelength (λ) of the sinusoidal grating that would best fit the spatial profile, this would predict a spatial-frequency bandwidth (Δf) of the order of $1/2\lambda$ — roughly one octave. As this is the order of magnitude of the bandwidths reported^{7,8} for simple cells, the receptive fields of these cells seem to represent a near-optimal solution to the problem of sampling optical images in *both* the frequency and the spatial domains. Their relatively wide bandwidth (low 'Q') suggests that at this stage the inevitable information-theoretic compromise is weighted in favour of spatial resolution; but to argue whether they are 'really' concerned with one or the

other would clearly be inept. The two aspects are logically complementary.

'Crucial' experiments?

In view of all this, it is surprising to find experiments that demonstrate the predictive power of Fourier analysis of stimuli still being presented as if they help decide between 'edge-detector' and 'frequency-filter' models of visual information-processing. To take a recent example, DeValois *et al.*⁹ attempted "to discriminate between two models of the response characteristics of simple and complex cells of the striate cortex" by comparing responses to gratings and chessboard patterns. A chessboard has its main spatial-frequency components oriented at 45° to the edges of its squares. If the cells of striate cortex were detectors of oriented bars or edges, it was argued, they should respond best when the edges of the chessboard squares lay at their preferred orientation. If they were spatial-frequency filters, they should prefer the edges to lie at 45° to it. In the event, DeValois *et al.* found the latter result, and claimed thereby to

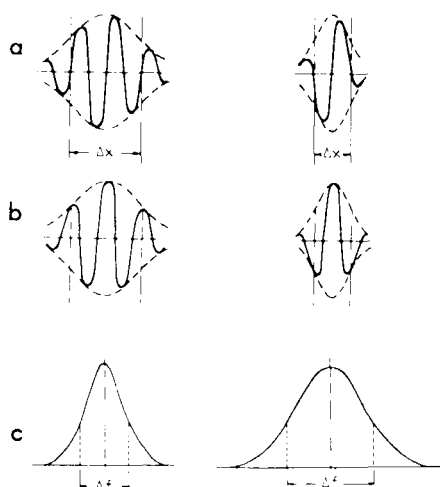


Fig. 1 Analogues in the spatial domain of Gabor's sampling functions of (a) 'sine' and (b) 'cosine' type, with (c) their corresponding spatial-frequency spectra (adapted from ref. 3). Note the reciprocal relation between the 'spreads' in space (Δx) and in spatial frequency (Δf).

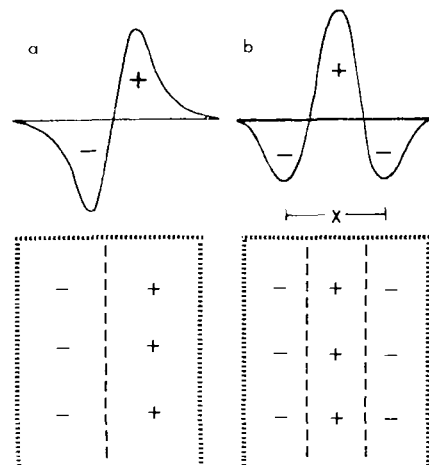


Fig. 2 Typical receptive field profiles (above) and idealized field maps (below) for simple cells with (a) one 'on' (+) and one 'off' (-) zone; (b) a central 'on' (+) and two 'off' (-) zones. The ordinate denotes the contributions to the excitability (or the firing rate) of the cell made by an optimally oriented light line, as a function of distance across the field. (For cells which are silent when unstimulated, such curves can be obtained by maintaining a steady background level of activity with an auxiliary stimulus.)

have confirmed the 'frequency-filter' hypothesis.

It is easy, however, to see how such results could be predicted without recourse to Fourier analysis. Considering first a single line of black squares (small compared with the separation of 'on' and 'off' zones) with a common diagonal (Fig.3a), everyone, including 'bar-detector' theorists, would expect the best response when the common diagonal lay parallel to the on-off boundary. Only if the squares were so large that one edge spanned the receptive field (Fig.3b) would the cell respond best when that edge was parallel to the on-off boundary. If we assume that light/dark gradients summate algebraically (even if non-linearly) over the receptive field, partial cancellation could be expected to occur in the case of Fig.3c, so that the response would be doubly position-dependent. Again, with a row of smaller squares as in Fig.3d, cancellation would be predicted¹⁰. With two diagonal lines of small squares (Fig.3e), Fig.2b would predict a maximal response for a critical separation (X) roughly equal to the spacing between 'off' zones — still with the diagonals parallel to the 'length' axis. With bigger squares (Fig.3f), the stimulus becomes a diagonal strip of chessboard pattern. The addition of further strips will introduce subsidiary maxima into the polar diagram at orientations giving a sufficient difference between the net excitation in the

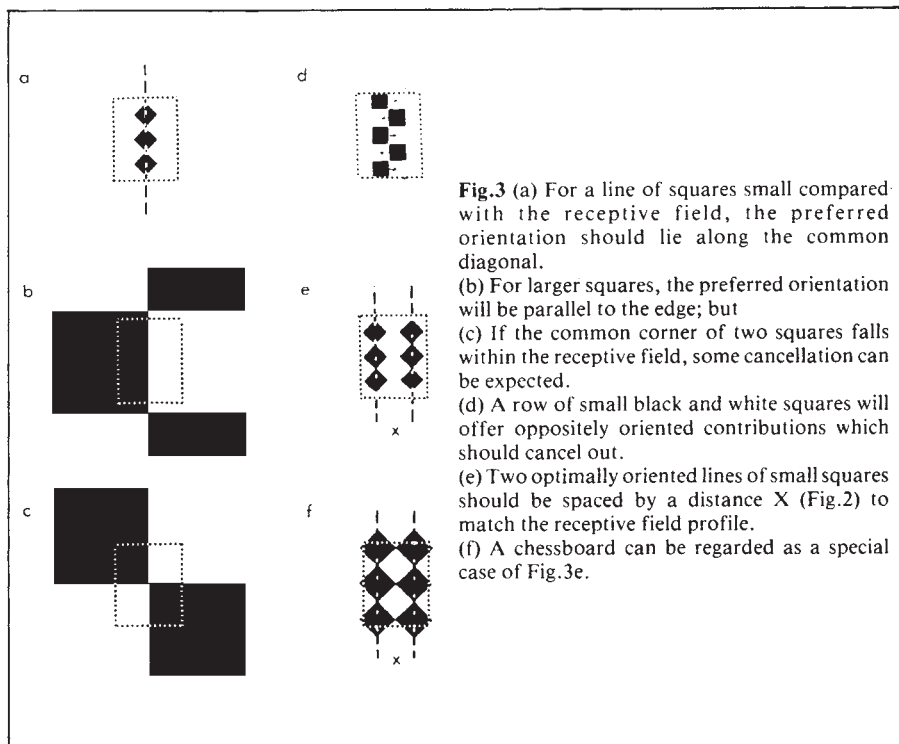


Fig.3 (a) For a line of squares small compared with the receptive field, the preferred orientation should lie along the common diagonal.

(b) For larger squares, the preferred orientation will be parallel to the edge; but

(c) If the common corner of two squares falls within the receptive field, some cancellation can be expected.

(d) A row of small black and white squares will offer oppositely oriented contributions which should cancel out.

(e) Two optimally oriented lines of small squares should be spaced by a distance X (Fig.2) to match the receptive field profile.

(f) A chessboard can be regarded as a special case of Fig.3e.

'on' and 'off' zones. Thus none of the results presented as crucial by DeValois *et al.* requires Fourier theory for its explanation.

The direct calculation of net excitation for various orientations and spacings, however, is laborious and inelegant. Fortunately, a mathematical technique is available to simplify and systematize such calculations. Its name — Fourier analysis. The fact that Fourier analysis predicts the polar diagrams speaks neither for nor against the theory that the cortex operates as a Fourier analyser¹². It shows only that the cell's response is (approximately) linear.

Empirical issues?

Is this then entirely a bogus issue, or are there genuine questions to be settled by experiment? Three empirical points may be noted:

Firstly, although all such cells are selective both for location and orientation and for spatial frequency, it is their relative *precision* in each domain that shows to what extent they could be useful (or useless) as filters. The frequency-selectivity of most simple cells is by engineering standards abysmal.

Secondly, the predictive success of Fourier analysis of stimuli does rule out some kinds of *non-linear* models of simple-cell function. For example, the evidence of DeValois *et al.*⁹ is clearly against any idea of 'edge-detectors' as cells so non-linear as to be insensitive to the polarity of luminance edges. (Talk of oriented edges as 'trigger features' for cortical cells may have contributed to this misconception.)

Thirdly, whether the firing of any single cell *signifies* to the CNS the presence of a specific feature is an empirical question of

cortical information-engineering design on which data are still scanty but thought-provoking. Hammond and MacKay¹³, for example, have found that the response of even simple cells to bars can be modulated by motion of a textured background, and have shown that most striate complex cells have different polar diagrams for motion of texture and of bars¹⁴. Again, recent work with multielectrode recording by Gerstein and Michalski¹⁵ suggests that more information may be represented by the pattern of temporal relationships between the firings of neighbouring units than by the firing patterns of any unit in isolation.

On both experimental and theoretical¹⁶ grounds, then, it seems timely to reconsider the possible roles of striate cortical cells in visual pattern recognition. As individual descriptors of the stimuli falling on their fields, they are imprecise or ambiguous in all the domains of space, frequency and velocity. As members of local cooperative assemblies they could collectively offer more exact descriptions. A third possibility, however, is that their job is not to provide a symbolic *description* of the visual field at all. The task of symbolizing the perceived world could well be a more central process¹⁷, which requires from the sensory system not pictures or descriptions but an array of selective clues to help it 'home in' on the appropriate symbolic representation. If so, we must be prepared to look for quite different kinds of link between striate cortical activity and pattern recognition. □

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Yeast genetics and molecular biology

from Terrance G. Cooper

LOUVAIN-LA-NEUVE was the site of the Tenth International Conference on Yeast Genetics and Molecular Biology. Twenty-four plenary presentations, eighteen evening workshops and over 200 posters allowed an exhaustive exchange of information about *Saccharomyces cerevisiae* and could lead one to conclude confidently that *S. cerevisiae* is now a favourite model for studying the structure and operation of eukaryotic cells.

RNA splicing was a major topic at the meeting and provided a focal point for studies of tRNA gene expression. The splicing system responsible for processing primary transcripts from eight or nine tRNA genes appears distinct from that processing mRNA. Mutants unable to remove intron sequences from *SUP4* tRNA primary transcripts had mutations in all portions of a recombinational fine structure map of the *SUP4* locus. This led to the conclusion that the entire three-dimensional structure of the intron-containing tRNA is probably important for recognition by the splicing enzyme system; this may act to provide a final opportunity for 'proof-reading' before activation of the newly synthesized tRNA.

Production of the *var1* polypeptide (a component of the small ribosomal subunit from mitochondria) may involve yet another example of RNA-RNA splicing. A genetic element has been identified which in some way specifies the size of a transcript, but is not contiguous with the DNA coding for it. The *var1* polypeptide is found in polymorphic forms ranging from 40,000 to 44,000 in molecular weight. A *var1* determinant, shown to be responsible for the polymorphism, is situated between the *ery* and *oli1* loci on the mitochondrial genome. However, complementation studies demonstrated that the *var1* determinant acts in *trans*. DNA sequencing studies revealed that the determinant does not possess apparent coding capacity (it is AT rich with no open reading frames) and is far too small to code for the *var1* transcript. Normal amounts of the *var1* transcript are found in petites containing only repeated copies of the *var1* determinants (these strains carry no other mitochondrial DNA) and hybridization experiments show a sequence homology of only 200 to 250 base pairs (bp) between the 1,900 to 2,000 bp *var1* transcript and this petite mitochondrial DNA. These observations have been used to suggest, by elimination, that the *VARI* gene is located in the nucleus; a directly testable hypothesis.

The search for the promoter region

occupied the attention of those trying to unravel the control of primary transcription. The results emphasized once again that control of gene expression in eukaryotes differs markedly from that in prokaryotes. A deletion ending 140 bp upstream from the ATG of the *his3* gene (transcription begins at position -45) resulted in loss of gene function as did a deletion ending 250 bp upstream from the ATG of the *cyc1* gene. Insertion of a *Ty1* sequence 161 bp upstream from the ATG of the *his4* gene (transcription begins at position -63) similarly eliminated transcription. In the case of the *gal* gene cluster, transcription of the contiguous *gal1* and *gal10* genes diverge from a single region of approximately 700 bp. It is not yet known whether or not transcription of the two genes is initiated from the same point. From these results it appears that a surprisingly large region might be required for effective promotion of transcription. However, it is important to note that deletions do not simply remove stretches of DNA. They also fuse the flanking sequences that remain and it may be important to ascertain the consequences of these fusions.

In a related area, four laboratories reported the structural characteristics of cloned genes (*adh2*, *car1*, *cyc2*, *dur1.2*) derived from mutant strains in which production of an enzyme was rendered constitutive by a mutation situated close to the structural gene. Constitutivity at these loci was observed in *MATa* and *MATa* haploid strains and *MATa/MATa* and *MATa/MATa* diploid organisms, but constitutivity was lost in *MATa/MATa* diploids. In each case analysed there appeared to be an insertion in or near the mutated gene. In two instances the inserted DNA was shown to be related to *Ty* sequences. Pertinent to these observations is the fact that RNA hybridizing to *Ty* elements has been reported to be the most abundant transcript expressed in parallel with mating-type specific functions, that is, such RNA is present in *MATa* and *MATa* cells, but is diminished in *MATa/MATa* diploid strains. While this raises the possibility that the constitutively expressed genes have been placed under the control of a new promoter as a result of the DNA rearrangement, transcripts derived from wild-type and constitutive *ADH2* mutants exhibiting the mating-type effect have been compared on northern blots and found to be the same size. It is also important to point out that insertion of *Ty* sequences into the 'control' region of a gene does not always result in its

constitutive expression or association of expression with mating-type functions. Two mutants unable to express the *his4* gene (*his4-912* and *his4-917*) were found to harbour *Ty* elements upstream from the transcribed sequences. A *His*⁻ phenotype was observed both in haploid and homozygous *MATa/MATa* diploid versions of these mutants.

Association of *Ty* sequences with a major proportion of the chromosomal rearrangements analysed so far prompted a search for other genetic elements associated with the influence of these mobile elements on genes in their proximity. This was done by selecting *His*⁺ revertants of the *his4-912* and *his4-917* mutants just discussed. One class of mutants (*spm2* and *spm3*) suppressed the effects of the *his4-917* mutation, but were incapable of suppressing the *his4-912* lesion. However, the reversion frequency of the latter mutation was increased 100-fold in *Spm2*⁻ and *Spm3*⁻ strains. Analysis of representative revertants revealed that recombination had occurred between the two directly repeated δ sequences flanking the inserted *Ty* element leaving behind a single copy of the δ sequence in place of the entire *Ty* element. Such revertants were found to be cold sensitive in a wild-type background, i.e. *His*⁺ at 37°C, but *His*⁻ at 22°C. In *Spm2*⁻ or *Spm3*⁻ strains cold sensitivity of the revertants was suppressed. A second class of mutations (*spm1*) suppressed the effects of both *his4-912* and *his4-917* mutations and cold sensitivity of the *his4-912* revertants. In formal terms these observations share several characteristics in common with the two-component *spm* control system of maize.

Several landmark events were identified for sporulation. Premeiotic DNA synthesis occupied the first 1.5–2 hours. Meiotic recombination was shown by direct measurement to occur at 4–6 hours, thus correlating with the pachytene stage. A novel and useful system for the manipulation of recombination-deficient mutants was presented. It has been difficult to diagnose recombination deficiency because of non-disjunction at meiosis I and the resulting spore inviability. These problems are circumvented with the *spo13* mutation. Strains harbouring such a mutation enter meiosis, undergo recombination and then, by-passing the reductional division of meiosis I, proceed

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directly to a single equational division of meiosis II. The latter division does not require recombination. Therefore, by coupling *spo13* to a putative recombination-deficient mutation, it is possible to follow recombination and sporulation independently.

Gene conversion was suggested to be the basic signal of meiotic recombination. The different types of segregation observed are then a function of how the recombination-heteroduplex is corrected. The *rad52* gene product was shown to be required for many recombination-associated processes including: mating-type switching, meiotic recombination, γ -radiation repair, meiotic and mitotic gene conversion. However, sister-strand exchange, plasmid integration into a chromosome and excision of an integrated plasmid occur normally in *Rad52⁻* strains. Mutants defective in the latter two processes were isolated and in one case found to be defective in all forms of mitotic recombination assayed. Transformation requiring plasmid integration (*Ars⁻*) was 25-fold lower in this strain. Also isolated were four classes of mutants (*cor*) exhibiting elevated frequencies of meiotic recombination. *Cor⁻* mutants were not sensitive to radiation and did not affect meiotic map distances or the frequency of initiating gene conversion. However, correction of heteroduplex intermediates generated during recombination was markedly reduced and the length of the correction tracts appreciably reduced.

The phenomenology and genetics of DNA repair is rather complex. Approximately 100 mutants have been isolated and characterized. This, however, may represent an overestimate of the genetic elements participating in repair, because allelism tests have not been completed. The results of characterization studies in many laboratories have led to the conclusion that five repair systems seem to exist. One mutagenic (error-prone) and two non-mutagenic systems are involved in the repair of radiation damage. Work with anti-mutator mutants suggests the existence of two mutagenic repair systems. One of these systems is identical with the mutagenic repair system associated with radiation damage. However, the second system appears to be independent of radiation damage. A fifth system appears to be needed for the repair of damage generated by the DNA alkylating agent, methyl methane sulphonate (MMS). This suggestion is based on the observation that some MMS-sensitive mutants are insensitive to radiation. The repair of psoralen-generated cross-linked DNA requires both non-mutagenic repair systems associated with repair of radiation lesions (the *rad3* and *rad51* systems). A new assay system has also been developed which permits one to follow the repair of DNA cross-links in 2 μ m circular DNA. This system should prove useful for studying DNA repair at the molecular level.

Finally, two reports describing very interesting types of DNA were presented. First was the centromeric DNA (*CEN3*) from chromosome III which was isolated on a 1.6 kb segment of DNA located near the centromere-linked *cdc10* locus. When present on a plasmid carrying a yeast chromosomal replicator (such as *ars1*), *CEN3* DNA enabled the plasmid to segregate as a chromosome both mitotically and meiotically. In meiosis the plasmid segregated 2+:2- rather than the 4+:0- characteristic of other unintegrated plasmids. In addition, plasmids containing centromeric DNA were found to be strikingly more stable. This stability would

be a most desirable characteristic in transformation cloning vectors. Such vectors are now being constructed. The second interesting type of DNA was reported to exist as a naturally occurring plasmid in *Kluyveromyces lactis*. This organism appears to harbour two linear DNA plasmids of molecular weights 5.4 and 8.4×10^6 respectively. They represent the first non-circular DNA plasmids reported in yeast or any other organism. Strains possessing the two linear plasmids were shown to kill both *S. cerevisiae* killer and sensitive strains. Non-killing *K. lactis* strains both sensitive and resistant to being killed themselves were also found.

Remote sensing of the oceans

from D.J. Webb

New techniques for remote sensing of the ocean surface have been tested in two cooperative experiments — the Marineland experiment off the coast of Florida and the West coast experiment off California — and the results reported in a recent issue of the *Journal of Geophysical Research* (85, 1980). The experiments were carried out in 1975 and 1977 and used centimetre radar, high frequency (HF) radar, optical and surface buoy measurements.

A variety of techniques have been successful in sensing properties of the oceans. Sound can be used at long range — sonar techniques give bottom topography, doppler shifts give the water velocity and changes in sound paths detect the movement of density surfaces. The use of electromagnetic sensors is limited by the high conductivity of sea water but comes into its own for monitoring the sea surface. Passive infra-red sensors can monitor sea surface temperatures and optical techniques can be used to measure the slope of ocean waves.

Recently, active systems have been developed, many using radar waves of between three and thirty cm wavelength. When directed vertically onto the sea surface the radar waves are scattered in a manner analogous to Sun glitter. In the SEASAT-1 radar altimeter the scatter can be used to give information on wave height and large-scale undulations of the sea surface¹. If altimeter and positional errors can be reduced below 10 cm such a system can also be used to detect tides and strong surface currents.

When radar is used at an angle of more than 30° from the vertical, reflection occurs mainly by Bragg scattering from the short surface waves. The height of these waves and thus the back-scattered cross-section depends on the wind field, the long waves they are riding on and possibly on the turbulence and surface tension of the

surrounding water. Any changes in these underlying parameters may be detected by the radar signal.

HF radar using wavelengths of tens to hundreds of metres may also be used. The radar signal is Bragg scattered by ocean waves of half the radar wavelength and interpretation of the returned signal is relatively simple. The method may be used to measure both the directional wave field and, from the doppler shifts, the surface currents, over distances of tens to hundreds of kilometres from the transmitter.

However, HF radar cannot be used from satellites because the signal is reflected by the ionosphere. Centimetre radars can be used but it is necessary to make sense of the complicated physics involved in the scattering process. It was this problem that led to the setting up of the 1975 and 1977 cooperative experiments.

In the first experiment², it was found that during a period of wave growth the power spectra for waves of between 1 and 5 seconds period did not depend on frequency to the power of -5, as the text books say, but instead the exponent lay between -3.5 and -4.5. The same experimental group using HF radar, with its accurate directional resolution, found evidence for the Phillips' mechanism of wave growth affecting waves of up to 25 m wavelength. These are rather larger than the catspaws on still water to which the theory is usually applied.

The HF technique was also used to study wave sheltering by islands³. A local Loran-A transmitter with a wavelength of 154 m was used and a directional aerial synthesized by driving a van at a fixed speed along nearly straight stretches of a suitable highway. The study showed a reduction in the wave energy behind the islands, but the

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residual energy was greater than could be explained by refraction or diffraction. The authors suggest that non-linear wave interactions may be responsible.

The island study also made use of synthetic aperture radar (SAR) using centimetre waves. A similar system used on SEASAT-1 had an all-weather resolution of 20 m, and around the UK showed swell waves off Northern Scotland, ships and their wakes in the Straits of Dover, disturbances produced by sand waves in the North Sea and internal tides propagating out into the Atlantic from the continental shelf edge.

The classical explanation of why swell waves are seen by such a system is that their orbital motion strains the short gravity waves and changes their amplitude. However, Wright and his co-workers⁴ found this explanation to be incorrect for typical ocean waves and suggest that it is the local wind field around the waves which is responsible for the observed signal.

Pawka and co-workers⁵ compared the directional wave spectrum obtained from SAR images with the spectra from a high resolution array of pressure sensors. They concluded that when waves could be imaged, the SAR gave useful information on the direction and directional spread of the waves.

Similar results were obtained by

McLeish *et al.*⁶. This group also found that, when normalized at the peak, the high frequency part of the SAR spectrum lay between the spectra obtained from wave height and orbital velocity measurements. The SAR system also showed swell being refracted by the Gulf Stream⁷ and swell being refracted and scattered by bottom topography as it approached the shore⁶.

When the same system was used in the much lower resolution scatterometer mode⁸, some gross changes were found on crossing the Gulf Stream. The authors conclude that this was due to changes in the surface stress due to the wind. This could arise because of the lower atmospheric stability over the Gulf Stream.

Finally, scatterometer measurements over a larger area were used to study how the back-scattered cross-section depends on the relative direction of the wind⁹. A much larger cross-section is observed when looking up or down wind than when looking across the wind, and a development of this technique was successfully used in the recent SEASAT satellite to measure the wind field over the ocean.

When the experiments were carried out the radar systems were all at an early stage of development. Since then the altimeter, scatterometer and SAR have all been successfully used in the SEASAT-1 satellite and commercial versions of the HF radar are being developed for coastal monitoring. Versions of these instruments are also planned for future satellites and one might predict that over the next 10 to 15 years the altimeter and scatterometer will become the standard instruments for measuring wave climate and winds over the oceans.

If bacteria are starved of methionine they can still respond to changes in the environment but they lose this ability to adapt. Mutants with aberrant behaviour, either tumbling continually (*Escherichia coli*, *che B*) or never tumbling (*E. coli*, *che X*) have also been found. The mutants can, however, be induced either to swim smoothly, or to tumble but fail to then adapt; *che X* mutants will continue to tumble for hours after repellent stimulation. The mutants proved defective in enzymes required to methylate or demethylate a specific group of inner membrane proteins.

These proteins, the methyl-accepting chemotaxis proteins (MCPs), are now known to be responsible for adaptation. Methyl groups are transferred to glutamyl residues on the MCPs from *s*-adenosyl methionine, in the presence of attractants, using a specific methyl transferase, creating a glutamyl methyl ester, or are removed in the presence of repellents using a methyl esterase. A high level of methylation is maintained as long as the attractant is present at a constant concentration, blocking the signal from the bound receptors. There are certainly two and probably three different groups of MCPs in *E. coli* and *Salmonella typhimurium* with molecular weights between 50,000 and 65,000, each cell probably having several hundred copies of each. Each group of MCPs responds to a specific set of chemosensory receptors which may be part of or able to bind to the MCPs and they provide parallel, almost independent pathways from their respective groups of receptors to the flagella.

Initially it was assumed that there were just two forms of MCP, one methylated and active, the other demethylated and inactive, controlling the function of a so-called 'tumble-generator'. However, on high-resolution polyacrylamide gels each MCP runs as a characteristic set of multiple bands.

While devising a method for methylating MCPs directly with labelled methionine in permeabilized cells, Paoni and Koshland (*Proc. natn. Acad. Sci. U.S.A.* **76**, 369; 1979) noticed that addition of attractants not only increased the intensity of MCP labelling on SDS gels but that the pattern of labelling within the bands changed, suggesting sequential methylation.

This observation has now been followed up in three papers published almost simultaneously and producing what seems to be at least part of the answer to the multiple banding on SDS gels and to adaptation, but showing that adaptation in bacteria is not the simple biochemical switch previously thought.

All three papers show that the multiple banding pattern on SDS gels is the result of sequential methylation of specific sites on the MCP polypeptide. Engström and Hazelbauer (*Cell* **20**, 165; 1980) used *in vivo* labelling and two-dimensional gel

Multiple methylation and bacterial adaptation

from Judith Armitage

THE ability of bacteria to sense, respond to and adapt to chemical stimuli provides a simple model system for the study of sensory transduction in general. It has been known for some time that protein methylation is a key factor in the process of adaptation to new chemical environments and now, a series of papers shows that methylation reactions are more complicated than had been previously thought and allow a much more flexible range of responses.

In an unchanging environment bacteria swim in a random fashion with periods of

smooth swimming interrupted by transient bouts of tumbling, caused by reversal of the direction of flagellar rotation. When a gradient of an attractant or a repellent is encountered, tumbling is suppressed when the bacteria are moving towards the attraction or away from the repellent resulting in overall migration towards positive or away from negative stimuli. A sudden change in attractant or repellent concentration produces similar behaviour; the small size of bacteria requires that they sense all gradients as temporal rather than spatial. However, in this case, after a short time bacteria adapt to the stimulus and resume swimming even though the chemical is still present.

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electrophoresis. The other two groups, DeFranco and Koshland (*Proc. natn. Acad. Sci. U.S.A.* 77, 2429; 1980) and Chelsky and Dahlquist (*Proc. natn. Acad. Sci. U.S.A.* 77, 2434; 1980) used recombinant genetics to introduce phage-directed cloned MCP-coding genes into *E. coli* and examined the labelling pattern of these MCPs in permeabilized cells. All the papers showed that stimulation with an attractant resulted in more heavily labelled faster-migrating bands, often with the appearance of a new fast band. However digestion of each band gave the same peptide pattern. At least three distinct peptides were shown to be methylated. Complete backbone labelling of the MCP by DeFranco and Koshland also revealed an additional slower-migrating band, corresponding to the unmethylated protein.

Sequential methylation of the MCP in response to an attractant, or demethylation in response to a repellent suggests that the number of sites methylated corresponds in some way to the number of receptor sites bound. The MCPs remain at the same level of methylation as long as a specific number of receptor sites is filled, inhibiting further

response to that chemical at that concentration, but allowing responses to changing levels of that chemical or to new stimuli up to saturation. This obviously allows flexibility within an otherwise limited system. Each MCP must, however, be able to interact with more than one receptor molecule at a time, or the stoichiometry of the receptor per MCP methylation must be greater than one.

Interestingly, both Hazelbauer and Dalquist showed that chemoattractants such as maltose and aspartate which produce different levels of bacterial response also gave different MCP banding patterns. This may help to explain why attractants with similar numbers of receptor proteins vary in their efficiency in eliciting a response.

The question remains of how the binding of a chemical to the receptor-MCP complex controls sequential methylation or demethylation. A recent paper from Adler's laboratory may provide the answer (Black, Hobson & Adler *Proc. natn. Acad. Sci. U.S.A.* 77, 3879; 1980). They show that fluctuations in internal levels of cyclic GMP may initiate adaptation. Mutants

showing defective adaptation also have defective cyclic GMP metabolism. Also, *in vitro* demethylation was inhibited by addition of cyclic GMP and cyclic GMP caused smooth swimming in the absence of chemoeffectors.

As yet the transducing signal between MCPs and flagella is unknown. It is almost certainly another function of the MCP but whether it is the release of a small molecule, possibly cyclic GMP as suggested by Adler, induced by a conformational protein change on binding, or a transient change in membrane potential or charge is not clear. It will also be interesting to discover whether bacteria are able to adapt to other tactic stimuli. Covalent modification of proteins has been found in several sensory systems in higher organisms, rhodopsin for example undergoes multiple phosphorylation when photostimulated and the methyl transferase system is involved in both stimulation of hormonal secretions in some higher organisms and leukocyte taxis. Sequential modification of proteins may therefore be a general biological phenomenon in signal transduction.

Photosynthesis in the Greek sunshine

from B. Halliwell and M. C. W. Evans

THE rapid advance in our understanding of the photosynthetic reaction centres has come about mainly through work on bacterial systems but knowledge of the plant reaction centres is now fast catching up. Components analogous to those in bacteria have been identified in photosystem II. Ke and Klimow (Yellow Springs, Ohio) have detected a pheophytin intermediary electron carrier, which was also reported in barley mutants by Nugent and co-workers (University College London). There is strong evidence that the subsequent acceptor is a quinone probably associated with an iron atom. Manipulation of the redox states of these components gives rise to EPR signals, absorption spectra and chlorophyll triplet states, indicating a mechanism much like that in purple bacteria (Rutherford, Urbana). Nugent *et al.*'s work on barley mutants also showed the importance of the donor to P680 as a source of the EPR signal of Photosystem II.

In Photosystem I, reviewed by Ke, EPR and endor measurements have shown that the pheophytin intermediate is replaced by a chlorophyll *a* monomer and the quinone acceptors by a group of iron-sulphur centres. Understanding of the purple bacteria is much more advanced, however. For example, Shuvalov (Puschino, USSR) and Parson (Washington) proposed a role for the B800 chlorophylls in the sub-picosecond primary photochemistry. Our knowledge of electron transfer outside the reaction centre is also best in the purple bacteria. A specific quinone involved in the oxidation of the Rieske iron-sulphur centre has been identified. This seems to exclude a Q-cycle system and requires a linear electron transport chain. However, a paired electron flow is indicated by the gating effect which can be observed in the oxidation and reduction of the secondary quinone acceptor (Q₂) and how these observations can be reconciled is not yet clear.

In chloroplasts the isolation of a cytochrome *bf* complex, analogous to the *bc* complex of mitochondria, suggests a similar mechanism in the quinone oxidation system. Since Z remains unidentified, however, the problem of a Q cycle

versus a linear electron chain is unresolved. Different schemes were proposed by Bouges-Bouquet (Paris), Rich and Bendall (Cambridge, England) and Hind (Brookhaven). The problem is further complicated by the conflicting requirements of cyclic and non-cyclic electron transport in chloroplasts. As discussed by Bendall, there is no definitive explanation of the function of the *b* cytochromes, particularly the two forms of *b*₅₅₉. Hence, while most participants at the Congress continued to accept the 'Z-scheme' of two light reactions in series, alternative arrangements have been proposed (for example, by Arnon at Berkeley, California). Indeed, there is room for some modification, since according to Bjorkman (Stanford) the relative numbers of photosystem I and II reaction centres and electron transport chains can vary with the growth conditions of the plant.

Perhaps the most disappointing part of the Congress was that dealing with water oxidation, which is still the least-well

*The Fifth International Congress on Photosynthesis was held at Halkidiki, (near Thessaloniki), Greece, from September 7th to 12th 1980, in appropriately sunny weather. The Congress dealt with all aspects of research in photosynthesis, from physiological ecology through carbon metabolism to the extreme limits of time resolution in the sub-picosecond photochemistry of the photosynthetic reaction centres.

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Interstellar media in other galaxies

from a correspondent

A recent international workshop at the European Space Agency's (ESA) Villafranca tracking station (Vilspa) near Madrid brought together those studying the interstellar medium in our own galaxy and those concerned with extragalactic problems, in particular the quasar absorption lines. Data from both the International Ultraviolet Explorer (*IUE*) satellite, which is controlled from Vilspa during its European observing shift, and Copernicus, as well as from ground based telescopes were discussed. The *IUE* has just begun making important observation of interstellar media and, with the demise of Copernicus at the end of 1980, will be the prime instrument for interstellar studies until the launch of Space Telescope.

Ted Snow (Colorado) reviewed work on the abundances of elements and their depletion in the interstellar gas as a result of intensive studies of particular sight-lines with the Copernicus ultraviolet spectrometer. While gas-phase abundances of some species, in particular carbon and oxygen, are little depleted and this sets limits on grain composition, the refractory elements are more highly depleted, consistent with either grain condensation in a high-density environment or preferential accretion of these species onto grain mantles by dynamic processes in interstellar clouds. Stuart Pottasch (Groningen) noted that, for a few species, the existence or degree of depletion depended on the cosmic abundances adopted and these were themselves not beyond controversy. Nolan Walborn (Cerro Tololo) reported some recent work on the dynamics of the interstellar gas in the η Carina nebula; relative velocities up to 550 km s⁻¹ are seen. Ed Jenkins (Princeton) discussed the presence of highly ionized gas in the plane of the galaxy. It appears that OVI lines

define a component with scale height above the galactic plane of ~ 300 pc and a velocity dispersion of 25 km s⁻¹. Their strength is correlated with reddening by dust. By contrast there is still some controversy whether the CIV and SiIV lines are truly interstellar. There seemed some evidence that the latter, at least, was in part circumstellar. Gordon Bromage (Chilton) claimed that NV lines were not seen in *IUE* data covering sight-lines to halo stars which rise out of the galactic plane so that the NV distribution looks similar to that of OVI with a scale height much smaller than that of the lower temperature CIV near 3 kpc. Max Pettini (Herstmonceux) noted, however, that he had one case where NV was seen in a halo star and he added that the three-time ionized carbon abundance may be lower in the plane than just outside it.

Klaas de Boer (Washburn) reviewed the recent exciting work by *IUE* on the galactic halo with emphasis on sight-lines to the Magellanic Clouds. There is some uncertainty if gas far from the plane co-rotates with the plane but, if so, the density falls by a factor of 10 over 10 kpc and one cloud covering 7 square degrees in the direction of the Large Cloud may contain 300,000 solar masses. There is component structure in OI and FeII but the SiIV and, possibly, CIV profiles suggested the halo has a smoother, less cloudy structure than the plane. There was, however, some discussion as to whether the direction to the Magellanic Clouds (where most of the *IUE* data exists) is typical of our galaxy. Claudine Laurent (Verrieres-le-Buisson) commented also that the existence of a similar halo around the Small Cloud was in doubt as she had data showing no CIV or SiIV in an SMC star which, unlike other cases observed to date, did not have its own HII region. Chris Blades (Chilton)

reported ground-based optical data from the Anglo-Australian Telescope (AAT) and found that the ratio of the neutral sodium column to dust reddening in the Small Cloud was 5 times smaller than that in our own galaxy.

Michael Penston (Vilspa) also showed AAT data of optical interstellar lines in other galaxies using galactic nuclei or supernovae as background probes. He confirmed earlier reports of CaII absorption in the Magellanic Stream. In discussion, the point was made that the (by necessity) moderate resolution on optical interstellar lines in other galaxies is similar in quality to the type of data that was available for our own galaxy in the 1940s and 1950s. While it is valid to analyse the galactic and extragalactic doublet ratios in similar ways and show that turbulent motions in interstellar media are generally characterised by velocity dispersions similar to that in our own galaxy, it was agreed that it is dangerous to deduce column densities from such material because large columns can be 'hidden' in components with low velocity dispersion. Alec Boksenberg (London) reviewed the current status of work on quasar absorptions lines. The absorption systems with metal lines can arise in galactic halos if these have radii of 100 kpc. The 'Lyman- α -only' absorption systems are not clustered like galaxies and suggest the presence of intergalactic clouds of primordial material.

On the theoretical side, Jo Silk (Berkeley) discussed other less direct evidence for intergalactic clouds including deduction from theories of galaxy formation. He proposed that slow self-regulating star-formation takes place in these clouds prior to their merging into galaxies as we know them. Joel Bregman (New York) described his 'galactic fountain' model of galactic haloes, in which hot gas of temperature T_0 at the base of the galactic corona rises, cools and turns into clouds which fall back towards the plane. Coronae of the right size are produced if T_0 is in the range one to six million degrees. A total mass of 10^{10} solar masses is required in these galactic haloes.

understood part of the electron transport system. Recent reports from Winget (Cincinnati) on reconstitution of this system using an isolated manganese-protein were not substantiated by other workers. For example, the reconstituted system described by Nakatani *et al.* (Imperial College London) requires a catalase-like protein rather than a manganese protein. Also, the NMR

measurements of Wyrdzinski and Govindjee (Urbana) that appeared to reflect changes in the redox state of manganese were challenged by Sharp and Yocum (Ann Arbor, Michigan). Nevertheless, materials for the solution of the 'water-splitting problem' are accumulating. Oxygen-evolving preparations with increased specific activity were described by Stewart (Cambridge,

England), inverted vesicles, that is, with the water oxidation system on the outside, were described by Albertsson (Lund), and Bishop (Corvallis, Oregon) has discovered mutants with defective water-oxidation systems. We cannot yet be 100 per cent sure, however, according to Metzner (Tubingen), that it is H₂O and not CO₂ that is releasing oxygen!

The sessions on chloroplast metabolism

dealt largely with the regulation of the Calvin cycle in C_3 plants, starch and sucrose synthesis, photorespiration and nitrogen metabolism. Few advances in our knowledge of C_4 plants were reported, although both Sugiyama (Shizuoka) and Hatch (Canberra) reported that regulation of the activation and deactivation of pyruvate-phosphate dikinase in the mesophyll cells is achieved by adenine nucleotides rather than thiol-dependent mechanisms. The pathway of chlorophyll biosynthesis in chloroplasts, particularly the route from glutamate to δ -amino-laevulinic acid, is becoming clearer. Chlorophylls *a* and *b* with modified side chains in the ring structure also occur in leaf tissues (Rebeiz, Urbana).

Regulation of the Calvin cycle is achieved by several mechanisms. One possibility proposed by Buchanan (Berkeley) and Anderson (Chicago) is a light-dependent increase in the activity of certain enzymes. In spinach chloroplasts, illumination generates reduced ferredoxin which produces, via a reductase enzyme, the reduced form of the thiol protein thioredoxin. Three types of thioredoxin (m_b , m_c and f) have been found in chloroplasts by Schurmann (Neuchâtel). Reduced thioredoxin *f* can increase the activity of some Calvin cycle enzymes assayed at physiological substrate and cofactor concentrations, and rapid rises in the activity of fructose and sedoheptulose diphosphatases on illumination of chloroplasts were reported by Leegood (Sheffield) and by Heldt (Gottingen). According to Charles (King's College London) in the case of fructose diphosphatase the rise in activity is due to an increase in the affinity of the enzyme for its substrate and cofactor rather than to a rise in V_{max} . When the light is turned off enzyme activities fall. How this is achieved has not been established. Heldt and others have shown that illuminated chloroplasts generate H_2O_2 , which deactivates the enzymes and may account for the lowered rate of CO_2 fixation in chloroplast preparations illuminated in the absence of added catalase. In the presence of catalase, however, the rises in enzyme activity on illumination are too fast to limit the rate of CO_2 fixation both before and after the induction period (Walker, Sheffield). Ribulose diphosphate carboxylase shows little light activation. Hence CO_2 fixation must be controlled by other means *in vivo*. This led Halliwell (King's College London) to propose that the thioredoxin system exists to protect the reduced, physiologically active forms of certain Calvin cycle enzymes from H_2O_2 generated *in vivo*, rather than as a regulatory mechanism. According to Heber (Düsseldorf), pseudocyclic photophosphorylation, generating H_2O_2 , must occur *in vivo* to correctly poise the redox state of the electron transport chain.

The major factors regulating CO_2 fixation seem to be the rise in pH of the

stroma on illumination, and possibly a light-dependent increase in the stromal Mg^{2+} ion concentration. Increases in stromal $[Mg^{2+}]$ from 1 to 3 mM have been reported in spinach chloroplasts, as discussed by Heldt, but Ben-Hayyim (Bet Dagan, Israel) has found no such increase in lettuce chloroplasts. There were some speculations that chloroplast Ca^{2+} ions, which powerfully inhibit fructose and sedoheptulose diphosphatases and protein synthesis, might be involved in turning off these reactions in the dark (for example, Jagendorf, Cornell).

Further developments in our knowledge of starch degradation in chloroplasts were reported by Stitt (Gottingen), who has isolated starch-loaded chloroplasts capable of degrading their starch at a physiological rate. Both amylase and phosphorylase activities are involved in this degradation (Beck, Bayreuth). Foyer (Sheffield) reported studies on the purified forms of the enzymes of sucrose synthesis. Claims by Garrett (Belfast) that isoenzymes of ribulose diphosphate carboxylase from diploid and tetraploid strains of ryegrass have different affinities for CO_2 and O_2 were fiercely contested, and more work is needed on this system.

The glycine-serine pathway is still regarded as the major pathway of CO_2

release during photorespiration in leaves, a view that was strengthened by the description of mutants of *Arabidopsis* with defects in various enzymes of the pathway (Ogren, Illinois). The ammonia generated during glycine decarboxylation is, according to Miflin (Rothamsted), assimilated by glutamine synthetase in the leaf cytosol. Other pathways of photorespiratory CO_2 release cannot be completely ruled out, however, since CO_2 evolution by direct glyoxylate decarboxylation has been reported in isolated leaf cells (Oliver, Idaho and Grodzinski, Ontario) and has been suggested by Ogren to occur at a high rate in an *Arabidopsis* mutant which cannot provide amino acids for the transamination of glyoxylate to glycine. Perhaps these alternative pathways are only important in nitrogen-deficient leaves.

The Rothamsted group also presented evidence that chloroplasts can synthesize amino acids of the aspartate family (Thr, Lys, Ileu, Met). It is now well established that ammonia assimilation in chloroplasts proceeds by the glutamine synthetase/glutamate synthetase route. Glutamine produced by cytosolic glutamine synthetase (see above) must enter the chloroplast for further metabolism by the ferredoxin-dependent glutamate synthetase. □

The (p, α) reaction

from P.E. Hodgson

THERE have recently been several studies of nuclear reactions emitting particles in an unstable or resonant state. These particles live long enough to act as a single particle during the reaction itself, but break up into two fragments soon after emission and long before they reach the detecting equipment. For example, it is possible for 8Be to be emitted from a nuclear reaction, but it is unstable and soon breaks up into two alpha-particles. The energy release is only 96 KeV, and so the alpha-particles continue to move in almost the same direction as the 8Be nucleus and are easily

identified by their strong directional and energy correlation compared with the single alpha-particles from other reactions. Another example of such a reaction is the (α, d^*) reaction with the emitted deuteron in the unstable singlet state at an excitation energy of 100 KeV.

It is particularly interesting to study such reactions as they extend the range of final states that can be reached and also provide an additional test of nuclear reaction theory. One reaction that was first identified a few years ago (*Nature* **266**, 121; 1977) is the (α, α^*) reaction with the outgoing alpha-particle excited to the unbound monopole state at 20.1 MeV. Now a detailed study of the corresponding (p, α^*) reaction has been made by Datta *et al.* at the University of Manitoba (*Phys. Rev. C* **21**, 1687, 1980). The excited alpha-particles decay into a proton and a triton, and these are detected by two counters as shown in Figure 1. The measurements were made with a 45.2 MeV proton beam on ^{12}C , and some of their results for the (p, α) and (p, α^*) reactions are shown in Figure 2.

The cross-sections of many nuclear reactions can be calculated by the distorted wave theory, and this may be applied to

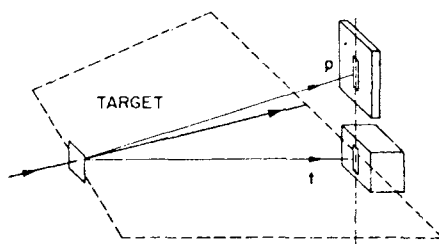


Fig. 1 Arrangement of detectors for the proton and triton from the breakup of excited alpha-particles.

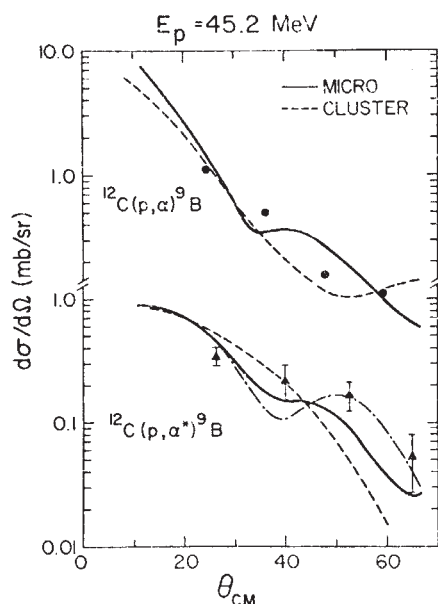


Fig. 2 Differential cross-sections for the (p,α) and (p,α^*) reactions on ^{12}C at 45.2 MeV compared with distorted wave calculations with the microscopic and cluster models. Y-axis, differential cross section in mb/sr; X-axis, angle in degrees. The dash-dot curve in the lower figure shows the result of another microscopic calculation using different distorting potentials. Each calculation is separately normalized to the experimental data.

these reactions, provided allowance is made for the unbound nature of the emitted particle. This is done by expressing its wavefunction as a product of a bound s-wave function and an energy-dependent Breit-Wigner resonance factor. There are two possible ways of treating the three particles picked up by the incident proton from the target nucleus. The simplest is to treat them as a cluster, and then the theory appropriate to a one-nucleon transfer reaction can be used directly with appropriate modification for the mass and charge of the transferred cluster. The other method is to treat the transferred nucleons individually, which is more correct but considerably more complicated. The cross-sections corresponding to calculations made in both these ways are compared with the experimental data in Figure 2. It is notable that on the whole the calculations treating the nucleons individually, called the microscopic model, fit the data somewhat better, although there is little to choose between them. Both calculations reproduce quite well the general features of the cross-sections and also the ratio between the cross-sections of the (p,α) and (p,α^*) reactions. There is some uncertainty connected with the choice of distorting potentials, but on the whole the agreement is sufficient to permit the conclusion that the (p,α^*) reaction is sufficiently well understood for it to be used in studies of nuclear structure. □

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Precambrian orogenies and polar wandering

from J.C. Briden

EVIDENCE that orogenic belts less than a few hundred million years old have formed (or are forming) at plate margins either at a continent/ocean interface or at a site of collision of two continents is overwhelming. It is consistent with the theory of a lithosphere made up of a small number of rigid plates in relative motion and encourages the interpretation of geology by extrapolation of the plate model far back in time. Extrapolation to more remote periods is uncertain especially because in Archaean times (beyond 3,000 Myr ago — the most remote parts of the geological record) evidence for rigid regions which might be the plate interiors is lacking. Hence notions have grown of intervals in which transitional tectonic regimes might have operated, with speculation that some or all older Precambrian orogenic belts do not mark former plate margins.

Palaeomagnetic studies have featured prominently in these arguments. The directions of permanent magnetism in continental rocks show slow systematic changes with age which can be depicted as paths of apparent wandering of the pole relative to each plate. For the past several hundred million years, the differences between the various polar wander paths are far more pronounced than any common component they may have, unequivocally demonstrating that the plates have been in motion relative to one another.

Returning to the Precambrian, much of the argument about the origin of orogenic belts relates to the degree to which polar wander paths from presently adjacent cratonic regions agree or disagree. The most serious problem is the impossibility of identifying the precise age of magnetization in Precambrian rocks, many of which have a complex history reflected in the frequent superposition of several palaeomagnetic components. Even where geochronological studies of the same rocks yield age estimates of various events in the rock history, uncertainties remain because events which leave palaeomagnetic imprint may not be synchronous with closure of isotopic systems in those rocks.

It has variously been argued (a) that the palaeomagnetic signatures from all fragments are essentially the same, being compatible either with true polar wander or coherent movement of a single super continent; (b) that they are all different and hence latter day plate tectonics was fully and exclusively operational as far back as

the palaeomagnetic record goes; and (c) that similarities and differences both exist, and hence some orogenic belts mark sites of continental collision and others do not.

The supercontinent model requires a single common polar wander path. This path can only be regarded as internally consistent if many inexplicable errors in magnetic age assignment have been made. It is also extraordinarily long, even allowing for the fact that it is almost certainly already smoothed by the failure to resolve superposed magnetic components in many suites of rocks. It implies a Proterozoic history utterly different from that of more recent times — a world in which polar wander or drift of a single supercontinent operated instead of a relative plate motion; in which deformation within that supercontinent occurred instead of plate interiors being rigid; and yet a world in which motions of the lithosphere were considerably faster than in more recent time.

On the other hand it is much too easy to simply link the sequence of palaeomagnetic data from different cratons, note that they are different, and conclude that cratons behaved like latter-day plates. The polar wander paths will inevitably look different because in almost every case each path will have been sampled inadequately and at quite different instants of time.

The middle-of-the-road model presently seems to find the most favour with palaeomagnetists because some of the very few comparisons of well determined polar wander paths from neighbouring cratons really do seem to agree, for period of times which post-date the deformation of the orogenic belts now lying between them. However, this is scarcely worthy of being called a model at all, because it fails to tell us which orogenic belts are merely compressions within pre-existing plates and which had a pre-history of large relative motion of their margins. It does not predict the style of motion when displacement has been large; nor can it rule out small openings and closings where they have been small.

In this issue of *Nature*, Onstott and Hargraves (page 131) argue for a fourth style of large motion (thus exhausting the alternatives and ensuring that at least someone is right!) with adjacent cratons sliding past each other along transcurrent

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fault belts with neither creation nor consumption of oceanic crust within those belts — though presumably it has to happen elsewhere in other parts of the plate-driving system. Parts of their argument are as weak as any that have gone before due to the inadequacies and paucity of the palaeomagnetic data on which they have had to rely. Nor is their interpretation unique: their data are equally compatible with a 'Wilson cycle' of an ocean which opened and subsequently closed. Yet their model serves as an important reminder that this transcurrent style of tectonics is possible and draws attention to evidence consistent with it in Proterozoic times — there are substantial examples in the Phanerozoic, particularly in the evolution of the Caledonian and the Hercynian belts

in Europe.

This element of relative plate motion is easily missed in tectonic analyses of orogenic belts as it is dependent on discovering a few surface traces of large faults parallel not only to the structural grain but most likely parallel to the grain of sedimentary facies as well. If one looks back at the palaeomagnetic evidence of different polar wander paths from Europe and North America published in the mid 1950s as evidence of the opening of the Atlantic, one would nowadays pick the individual data to shreds; few if any would be accepted as adequately confirmed palaeomagnetic directions by current criteria. Yet the correct answer could be seen, and was indeed seen, in the systematic deviation of sets of (internally suspect)

data. The same may be so for Onstott and Hargraves' contention of large-scale transcurrent motions. As in the Phanerozoic the confirmation came from elsewhere than continental palaeomagnetism (in dated magnetic anomalies, transform fault interpretation, and the whole range of sea floor spreading data), so for the Proterozoic other disciplines may resolve the problem. The critical lines of inquiry are likely to be careful tectonic analysis of structures in plan, and seismic 'profiling' right through the lithosphere to give structure in cross-section. Mesozoic to present geological experience tells us that there are enough distinctions between major transforms and sites of destruction of major oceans or back arc basins for solutions to this problem to be attainable.

100 years ago



Mongoloid types, Indo-China. King and Queen of Siam.

MONGOLIAN TYPE

from A. H. Keane

We are all familiar with the essential characteristics of the Yellow or Mongolian division of the human family.

They correspond substantially with those we see in the ordinary Malay type, in Java, Bali, Madura, many parts of Sumatra, round the coast of Borneo, and in the peninsula of Malacca. The true aborigines of this region were the Negritos; consequently the Malays, like the pre-Malays or Caucasian Indonesians, are here intruders. Intruders from where? Obviously from where the type exists, the neighbouring Indo-Chinese peninsula. What then becomes of the Malay as a primary division of mankind? As such it can no longer be recognised in anthropology, and must sink to the position of a mere variety of the Mongol type. The so-called true Malay or typical Malay is essentially a Mongolian, and the likeness between the two has not failed to strike all careful observers. "The Malayan race," says Wallace, "as a whole undoubtedly very closely resembles the East Asian populations from Siam to Manchuria. I was much struck with this, when in the Island of Bali I saw Chinese traders who had adopted the costume of that country, and who could then hardly be distinguished from Malays; and on the other hand I have seen natives of Java who, as far as physiognomy was concerned, would pass very well for Chinese." Hence De Quatrefages rightly rejects the claim of the Malays to be regarded as a fundamental type. "All polygenists," he remarks, "have regarded the Malays as one of their *human species*; many monogenists have considered them as one of the principal races. I showed long ago that in reality they are only a mixed race in which white, black, and yellow elements are associated."

The last clause of this sentence gives the true solution of the problem. The inhabitants of Malaysia consist not of one, nor even of three distinct races, but of three races variously intermingled, the yellow or Mongolian, and the white or Caucasian chiefly in the west, these two and the black or Papuan chiefly in the east. As the fusion of yellow, white, and black produces the so-called "Alfuros" in the east, so the fusion of yellow and white produces the so-called Malays in the west. The more the yellow prevails the nearer do the Malays approach the Mongol type; the more the white prevails the nearer do they approach the Caucasian type, until in some places they seem to be no longer distinguishable from the Mongols, in others from the Caucasians." From *Nature* 23, 13 January, 250, 1881.

Fallacies of lifestyle cancer theories

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Self-interest of the chemical industry apart, the lifestyle theory of cancer causation is held to reflect misinformation and 'inactive conservatism'.

PETO'S article¹, purporting to be a review of the book *The Politics of Cancer*², is largely a restatement of the lifestyle theory of cancer causation. This theory postulates that if you get cancer it is essentially your own fault, and that the causal role of past involuntary exposure to environmental and occupational carcinogens is trivial. Not surprisingly, the lifestyle theory has emerged as the major professed basis of the chemical industry's objections to the regulation of its carcinogenic products and processes³. As an enthusiastic proponent of this theory, Peto asserts that smoking-derived and fat-associated cancers "collectively account for more than half of all cancer deaths." As a corollary, of Peto's emphasis on lifestyle factors, he denigrates the role of occupational and environmental carcinogens and the need for their effective regulation, claiming that there has been no recent increase in cancer mortality rates other than that due to smoking. We shall demonstrate that there is scant scientific basis for the lifestyle theory, and that it is in fact contradicted by a substantial body of published evidence.

Cancer rate trends

Peto justifies his emphasis on lifestyle factors by dismissing evidence for recently increasing cancer rates, apart from "that due to the massive effects of smoking on lung cancer". However, there is substantial evidence to the contrary. Standardized cancer death rates, adjusted to the 1940 age structure of the total United States population, show a progressive overall increase of about 7% from 1935 to 1970 (ref. 4) despite marked reductions of stomach cancer rates for unexplained reasons and of cervix cancer rates for reasons including the frequency of elective hysterectomy for non-malignant disease and the success of screening programmes. These trends are consistent with standardized mortality data for the United States (Table 1)⁵, where they are even more marked in black males, and with crude mortality data for the United Kingdom (Table 2)⁶. The overall rate of increase in US cancer mortality in the 7-year period from 1969 to 1976 (5.5%), adjusted to the 1970 age structure, is substantial and comparable with that for the preceding 35 years, 1935 to 1970 (7%). The overall increase in incidence rates is even more marked than mortality rates in the past decade, involving a wide range of organs besides the lung (Table 3)⁵. Moreover, the increase in incidence for all sites is comparable with that when lung cancer is excluded (Table 4)^{7,8}.

Reliance on overall age-adjusted incidence or mortality rates alone is simplistic, as such rates can mask steep increases in organ-specific cancers in high risk population subgroups, such as asbestos insulation workers or menopausal women treated with oestrogen replacement therapy. The overall probability, at today's death rates, of a person born now getting cancer by the age of 85 is 27% for both men and women; this is increased from the 19% for men and 22% for women born in 1950 (ref. 9). Furthermore, recent cancer rate trends reflect exposures and events beginning some 20 or 30 years ago, when the production of synthetic organic chemicals was relatively trivial compared with the present levels. The production of synthetic organic compounds in the United States in 1935, 1950 and 1975 was about 1, 30 and 300 billion pounds per annum, respectively¹⁰; sharp increases have also been observed for a wide range of derived industrial products such as chlorinated hydrocarbon solvents, plastics and resin materials, and of industrial

carcinogens, such as vinyl chloride and acrylonitrile. It is reasonable to anticipate that greater production has been paralleled by increased exposure of increasing numbers of both the workforce and the general public, which is likely further to accentuate increasing trends in cancer rates. It must also be recognized that before the 1976 Toxic Substances Act, which the chemical industry so effectively stalled for so long¹¹, there were no requirements for testing chemicals before their introduction into commerce (with the exception of special-purpose legislation for drugs, pesticides and food additives). Thus, the overwhelming majority of industrial chemicals now in use have never been tested for chronic toxic and carcinogenic effects, let alone for ecological effects.

Role of smoking

As emphasized in *The Politics of Cancer*¹ (p. 178), "Smoking is the single most important cause of lung cancer, as well as of cancer at other sites, chronic bronchitis and emphysema, and cardiovascular diseases". Less well appreciated by lifestyle advocates is that overemphasis on smoking is widely used to divert attention from occupational causes of lung and other cancers. Of the approximately 100,000 annual lung cancer deaths in the United States, at least 20% occur in nonsmokers. It is relevant that lung cancer death rates in nonsmokers approximately doubled¹² from 1958 to 1969, an increase maintained since. Furthermore, the role of occupational exposure to carcinogens was not recognized in most of the classic epidemiological studies which linked lung cancer with smoking. This led to overestimation of the contribution of smoking compared with occupational risks or to their possible interactions.

Table 1 Age-adjusted cancer mortality rates per 100,000 US population for selected sites by sex and year 1969–76, and average per cent change⁵

Site	Sex*	Mortality rate per 100,000		Average % change 1969–76	
		1969	1976	Annual	7-Year
All sites	WM	195.0	210.2	0.9	7.8
	WF	129.0	133.8	0.5	3.7
Stomach	WM	10.6	8.7	-2.9	-17.4
	WF	5.3	4.1	-3.6	-22.6
Colon	WM	18.7	20.7	1.3	10.7
	WF	16.2	16.5	0.0	1.9
Rectum	WM	6.9	5.6	-3.0	-18.8
	WF	3.9	3.2	-3.1	-17.9
Pancreas	WM	11.0	11.0	0.0	0.0
	WF	6.6	6.8	0.3	3.0
Lung	WM	55.0	66.7	2.6	21.3
	WF	10.2	17.8	7.6	74.5
Melanoma	WM	2.0	2.6	4.0	30.0
	WF	1.4	1.6	0.8	14.3
Breast	WF	26.2	27.2	0.3	3.8
Cervix	WF	5.5	3.9	-4.9	-29.1
Uterus	WF	4.6	4.2	-1.7	-10.5
Prostate	WM	19.0	21.0	1.2	8.7
Bladder	WM	7.1	7.5	0.6	5.6
	WF	2.1	2.0	-1.4	-4.8
Kidney	WM	4.3	4.5	0.6	4.7
	WF	2.0	2.1	0.7	5.0
Leukaemia	WM	9.4	9.2	-0.4	-2.1
	WF	5.7	5.2	-1.7	-8.8

For age adjustment the 1970 United States population was used as standard.

* WM, white male; WF, white female.

Table 2 Crude cancer mortality rates per 100,000 population for selected sites by sex and year, England and Wales, 1971–77, and average per cent change⁶

Site	Sex	Mortality rate per 100,000		Average % change 1971–77	
		1971	1977	Annual	6-Year
All sites	M	265	283	1.1	6.8
	F	215	233	1.3	8.4
Stomach	M	30	28	-1.2	-6.7
	F	21	19	-1.7	-9.5
Large intestine and rectum	M	30	32	1.1	6.7
	F	34	35	0.4	2.9
Respiratory	M	106	112	0.9	5.7
	F	22	29	5.3	31.8
Breast	F	45	47	0.7	4.4
Uterus	F	15	16	1.1	6.7
Prostate	M	17	19	1.9	11.8
Bladder	M	12	12	0.0	0.0
	F	4.1	5.1	3.7	24.4
Leukaemia	M	7.0	7.2	0.4	2.9
	F	5.5	6.0	1.5	9.1

Table 3 Age-adjusted cancer incidence rates per 100,000 US population (whites) for selected sites by sex and year, 1969–76, and average per cent change³

Site	Sex	Incidence rate per 100,000		Average % change 1969–76	
		1969	1976	Annual	7-Year
All sites	M	346.6	374.0	1.3	7.9
	F	271.5	301.2	2.0	10.9
Stomach	M	15.4	12.6	-2.3	-18.2
	F	7.1	5.6	-3.7	-21.1
Colon	M	34.5	36.9	1.5	7.0
	F	30.6	31.4	0.7	2.6
Rectum	M	17.5	19.4	1.3	10.9
	F	11.1	11.4	1.2	2.7
Pancreas	M	12.1	11.5	-0.5	-5.0
	F	7.5	8.0	0.9	6.7
Lung	M	70.6	77.8	1.4	10.2
	F	13.3	23.7	8.6	78.2
Melanoma	M	4.4	6.8	6.8	54.5
	F	4.1	6.1	6.2	48.8
Breast	F	73.9	83.5	1.8	13.0
Cervix	F	16.0	10.6	-5.9	-33.8
Uterus	F	22.6	31.2	5.9	38.1
Ovary	F	14.9	13.6	-0.4	8.7
Prostate	M	59.0	68.6	2.3	16.3
Bladder	M	23.8	26.4	2.3	10.9
	F	6.3	7.3	2.5	15.9
Kidney	M	9.0	9.6	1.2	6.7
	F	4.3	4.8	1.3	11.6
Leukaemia	M	13.2	13.1	-0.2	-0.8
	F	8.0	7.1	-1.0	-11.3

The 1970 population was used as standard for age-adjustment.

Thus, "we are unable to say how much of the risks attributed to cigarettes is a 'pure' cigarette risk and how much is cigarette times another, possibly on the job hazard"¹⁸. Moreover, smoking and occupation are confounded variables, smoking among men being more prevalent in 'blue-collar' workers than in professional and managerial classes¹³. Occupational causes of lung cancer include asbestos, radon daughters, nickel ores, chromium, arsenic, beryllium, mustard gas, vinyl chloride and bischloromethyl ether, apart from incompletely identified carcinogens in a wide range of industries such as rubber curing, tanning, steel (coke ovens), foundries, automobile, and petrochemicals. Thus, lung cancer rates in asbestos insulation and topside coke oven workers are as much as 10 times greater than general population rates.

Underestimation of the role of such occupational carcinogens has been assisted by the fact that lung cancer mortality rates, based on the International Classification of Diseases, fail to distinguish pleural mesotheliomas from lung cancers; there is evidence of substantial under-reporting of mesotheliomas (by about 75%) in high risk groups¹⁴, and even more so in occupations, such as automobile mechanics, where asbestos exposure has not been well recognized. There is a further lack of distinction between lung cancers of different histological types, some of which, such as adenocarcinomas, are less likely to be

due to smoking than to occupational carcinogens^{15,16}. "In several instances where the risk of bronchogenic carcinoma has been shown to be increased among occupationally exposed groups, there has been an accompanying shift in the distribution of histologic types of tumours", away from the small-cell undifferentiated and squamous cell carcinoma of the lung, the principal types whose frequency is increased by smoking, in the direction of other types, particularly adenocarcinoma¹⁶. "This (shift) has been noted among metal miners, uranium miners, copper smelter workers, vinyl chloride polymerization workers, chloromethyl methyl ether production workers, and mustard gas manufacturers" (ref. 16).

Possible variations of smoking patterns fail to account for the marked excess in US lung cancer rates identified in specific occupational exposures, particularly among ethnic minorities and migrants from southern states¹⁷. A further challenge to the dominant role ascribed to smoking seems to be provided by observations that the risk of lung cancer in certain occupational groups, such as American Indian uranium miners¹⁸, Swedish zinc-lead miners¹⁹, mustard gas workers²⁰, copper smelters exposed to arsenic²¹, and chloromethyl methyl ether workers²², is about as high among nonsmokers as smokers, although the latency period is reduced in smokers, suggesting a possible promotional effect of smoking. It appears that the relative risks of lung cancer for smokers as against risks for nonsmokers may have been overestimated, particularly in less than lifetime studies¹³. Variations in smoking do not account for geographic excesses in lung cancer rates in US males and females, which overall reflect proximity of residence to petrochemical and certain other industries^{23,24}; there are also data showing associations between levels of atmospheric carcinogens and lung cancer mortality rates²⁵. It may be noted that a report²⁶ from Peto's own institution demonstrates that the correlation coefficient between lung cancer and smoking internationally explains only one-third as much of the variation as does the correlation between lung cancer and solid fuel consumption (0.4 versus 0.7; $r^2 = 0.16$ versus 0.49).

Overemphasis on the carcinogenic effects of smoking, and ignoring or discounting the role of occupational and other exposures, is extended by Peto and others to cancers of the

Table 4 Changes in US cancer incidence rates from 1970 to 1975^{7,8}

Groups	Average % increase in incidence rates, 1970–75			
	Cancers of all sites		Cancers of all sites except lung	
	Annual	5-Year	Annual	5-Year
White male	0.9	4.7	0.9	4.6
Non-white male	2.3	11.9	2.7	14.3
White female	2.2	11.6	1.8	10.2
Non-white female	6.1	34.6	5.7	32.2

Table 5 International correlations between breast, colon and liver cancers and possible aetiological variables²⁶

	Correlation coefficients			
	Consumption of fat	Consumption of animal protein	Gross National Product	Total energy production
Breast cancer				
Incidence	0.79	0.77	0.83	0.70
Mortality	0.89	0.83	0.72	0.60
Colon cancer (M)				
Incidence	0.74	0.74	0.81	0.68
Mortality	0.85	0.86	0.77	0.69
Colon cancer (F)				
Incidence	0.78	0.80	0.82	0.67
Mortality	0.81	0.84	0.69	0.62
Liver cancer (M)				
Incidence	-0.49	-0.59	-0.42	-0.25*
Liver cancer (F)				
Incidence	-0.59	-0.67	-0.53	-0.31*

* 'Liquid energy'.

bladder and pancreas which are variously characterized as related to or caused by smoking^{27,28}. However, the relative risks for these cancers are several times less in smokers compared with nonsmokers than is the case for lung cancer. Excess bladder cancer rates have been identified in several occupational categories, including rubber, paint manufacturing and textile dyeing workers²⁹, and among residents in highly industrialized counties³⁰, particularly those with large chemical industry complexes³¹. Excess pancreatic cancer rates have also been reported in various occupations including steel and metal workers³² and organic chemists.

Recognition of the important role of occupational exposures in lung cancers previously ascribed, exclusively or largely, to smoking in no way detracts from the recognition, emphasized in *The Politics of Cancer*, that the impact of smoking constitutes a "national disaster". There is no basis for regarding the smoking/lifestyle and occupational theories as mutually exclusive, particularly as these exposures may operate interactively. Furthermore, lifestyle is a somewhat misleading rubric for smoking as it restrictively implies voluntary personal choice. Placing responsibility for personal choice of an addictive lethal habit on young teenagers, the fastest growing group of new smokers, seems inappropriate. Failure to control smoking reflects a wide range of political and economic constraints, including massive press advertising by the industry which omits the word 'death' from the guarded small print warning of danger, massive revenues to federal, state and local government from tobacco taxes, federal subsidies to the industry and unwillingness of governments to increase tobacco taxation or to develop incentives to tobacco farmers to diversify. It is also important that the industry has moved to open up massive new markets with high-tar cigarettes in less developed countries, where the population is poorly informed on the hazards of smoking.

Role of diet

Lifestyle proponents are on less sure ground when they bracket diet, excess fat and overnutrition with smoking as the causes of the majority of cancer deaths. This claim is based largely on international correlations between consumption of total fat and rates for cancer of the breast and colon²⁶; however, such correlations by themselves are not proof of causality. Similar correlations were found, in the same study from Peto's institution, between breast and colon cancers and other variables, such as Gross National Product and consumption of animal protein, which also appear to reflect industrialization²⁶ (Table 5). Furthermore, "epidemiologically, the case against fat is weak because there are populations that have a high fat intake and little bowel cancer..."³³. Of two case control studies on the association between diet and breast cancer, one found no effect³⁴ and the other found trivial effects of fat and caloric intake, concluding that "... recommendations of major dietary modification as a possible preventive measure for breast cancer are clearly premature"³⁵.

Equally unconvincing are the studies, cited by Peto as corroborative evidence on the experimental effects of diet, which were largely concerned with the influence of fat on the incidence of tumours induced by chemical carcinogens and ionizing radiation, and the influence of caloric intake on the incidence of spontaneous and induced tumours. Not only were different variables defined in the animal and human studies—per cent fat in the diet and total dietary fat, respectively—but increasing fat levels in the animal experiments were associated with increased incidence of skin, liver and breast cancers, whereas the reported correlations between fat consumption and liver cancer mortality are negative for both men and women (Table 5). Moreover, these experiments often failed to differentiate between variations of total dietary fat and caloric intake in test animals and to adjust caloric intake in controls to reflect dietary fat variations in test animals; the magnitude of the variations in fat and caloric intake required substantially to influence the incidence of induced and spontaneous tumours in experimental animals is

generally far in excess of the dietary differences observed among the various human populations studied³³. These experiments invariably failed to adjust the intake in controls of fat soluble carcinogens, present in fat as accidental environmental contaminants, to reflect variations of fat intake of test animals.

Peto's claim for the causal role of dietary fat in human cancer overstates the conclusions of those cited as the basis for his claims. Armstrong and Doll²⁶, for instance, merely suggest that dietary fat levels may influence the incidence of colon and breast cancers, without asserting causality. Doll considers that diet may act by modifying the incidence of tumours induced by carcinogens or by acting as a vehicle for exogenous carcinogens³⁶—a suggestion also made in *The Politics of Cancer* which Peto dismisses as "implausible". Carrol concludes that "although caloric intake may be a factor in human carcinogenesis, it does not appear to offer a practical approach to the problem"³⁷. As recognized by current concepts on the multifactorial aetiology of cancer, there is a substantial probability that a wide range of influences, diet and other lifestyle factors included, modify individual responses to carcinogenic agents. To ascribe causality to any particular modifying factor requires a degree of scientific evidence that has not yet been presented for dietary fat.

Role of occupation

Peto associates himself with the insistence by the chemical industry² and other lifestyle proponents that occupational exposures account for about 5% (refs 38–40) or "a very small proportion"⁴¹ of all cancers. This view is based on ascribing given percentages to known or alleged lifestyle factors, including smoking, fatty diet and sunlight, leaving a small unaccounted for residue to which occupational factors are arbitrarily assigned by exclusion. The authors of this simplistic hypothesis compensate for its tenuous basis by reliance on 'educated estimates' and by making circular references to each other, often by 'personal communication', as the responsible authority.

However, there are problems with such 'guesstimates'. First, they fail to consider the multifactorial aetiology of cancer and the role of multiple causal agents, such as asbestos and smoking⁴²; thus, the summation of known causes of cancer should properly exceed 100%. As one of the lifestyle authors recently stressed³⁶, "there is now strong evidence to suggest that the risk of cancer is commonly increased by interaction of two or more factors". Second, current cancer rates reflect exposures 20 to 30 years ago, when production levels of occupational carcinogens were a small fraction of the present; such estimates should thus now be adjusted to reflect increasing numbers of workers exposed. Third, the authors of these guesstimates failed to consider the very limited nature of the data base on exposure to occupational carcinogens. Nor have they at any stage protested or even commented on the persistent refusal of the chemical industry to make such critical data available. In the absence of exposure data, it is even less clear how the 'lifestylers' confidently arrive at their estimate of less than 5%.

Rather than addressing himself to such problems, Peto dismisses recent estimates of the importance of occupational carcinogens in a report by the US Public Health Service⁴³ as exaggerated, unsound and unreasonable. This report, prepared by nine named and internationally recognized experts in cancer epidemiology, statistics and carcinogenesis from three federal research agencies, is based on a National Occupational Hazard Survey which between 1972 and 1974 surveyed nearly 5,000 workplaces chosen to provide a cross-section of industry in the United States. The report estimated the total number of workers exposed to asbestos, nickel ores, chromium, arsenic, benzene and petroleum fractions, including aromatics. The excess cancers attributable to each of these carcinogens were derived by multiplying the number of exposed workers by known risk ratios and subtracting the "normal incidence" of the cancer.

The report concluded that "as much as 20% or more" of cancers in the near term and future may reflect past exposure to the six carcinogens considered. The uncertainties and

limitations in these conclusions, including the possibility that exposures and risk ratios may have been overestimated in some instances, were clearly stated in the report, as were other considerations including the multifactorial aetiology of cancer, and the role of lifestyle factors and their possible interactions with occupational exposures.

The possibility that this government report underestimates rather than overestimates the role of occupational exposures, for several reasons some of which are recognized in the report, has not been considered by its denigrators, including Peto. First, the calculations in the report ignore the role of radiation and of some ten epidemiologically recognized occupational carcinogens, other than the six considered. Second, the risk ratios considered may be artificially low as they were largely derived from less-than-lifetime epidemiological studies, which may thus underestimate the true risk in view of the long latencies commonly involved. Third, the report does not consider the many statistical and methodological constraints common to most occupational epidemiological studies⁴⁴ such as relatively small numbers of workers in many locations, changes in exposure patterns over time due to employee turnover, plant shutdown, process and production changes and changes in management, all of which lead to fragmentation of health and exposure records, access to which is often restricted by industry. Fourth, the estimates fail to take account of the many chemicals recognized as carcinogenic in animals for which there are no exposure or epidemiological data. Thus, of 442 chemicals and industrial processes recently evaluated by the International Agency for Research on Cancer (IARC), epidemiological data are available for only 60 (14%), although evidence of experimental carcinogenicity was considered to be sufficient for 143 (32%)⁴⁵. Fifth, the estimates exclude high risk occupations with incompletely defined carcinogens, such as the steel, rubber and tanning industries. Sixth, the estimates do not adequately reflect conditions in small business where exposure levels are likely to be higher than in major chemical companies. Seventh, the report does not reflect major increases in the production of the occupational carcinogens it considered such as benzene, with the likelihood of recently increasing exposures. Eighth, the study examined only a limited number of sites, excluding cancers such as skin and bladder which are known to be occupationally related. Finally, the estimates neglect the possible role of fugitive point-source emissions of industrial carcinogens as causes for the excess of overall and organ-specific cancers, including lung, bladder, colon, pancreas and breast, in residents of certain highly industrialized counties.

This government report has received extensive support from various expert bodies, such as the Toxic Substances Strategy Committee, whose position has been endorsed by 17 federal

agencies, and international groups, such as the International Labor Organization, and the US and British trades union. The report has also received additional support in the critique of two consultants to the chemical industry's American Industrial Health Council which concluded that "... the full range (of total cancer attributable to occupational exposure) using multiple classifications may be from 10 to 33% or perhaps higher if we had better information on some other potentially carcinogenic substances The annual number of cancer deaths attributable to asbestos is in the range from 29,700 to 54,000, which corresponds to a percentage range of the total cancer of 7 to 14% Any argument over these numbers cannot detract from the fact that asbestos exposure was, as the authors (of the Government report) state, a major public health disaster We also believe that reduction of exposure to carcinogens in the course of employment can certainly be expected to affect major reductions in the frequencies of occurrence of cancer and is one of the most promising applications of preventive medicine"⁴⁷. The American Industrial Health Council failed to release this critique until the record of the recent Occupational Safety and Health Administration hearings on regulation of occupational carcinogens closed.

Finally, there is no basis whatsoever for recent unsubstantiated allegations by Peto and others that all or most authors of the government report have disowned or rejected it or its conclusions (K. Bridbord, M. Schneiderman and A. Upton, personal communication). It should be further emphasized that this 50-page report was prepared as a government document specifically for inclusion in public hearing records, and not for submission to a scientific journal.

Conclusions

Cancer is a disease of multifactorial aetiology to which occupational exposure and smoking can contribute importantly, sometimes interactively. There have been substantial recent increases in cancer rates which cannot be accounted for by smoking alone. Smoking is the major lifestyle factor of importance in cancer, and evidence for the causal role of other lifestyle factors, particularly diet, is slender. The role of lifestyle factors has been exaggerated, by those with an economic or intellectual investment in this theory, by largely excluding involuntary exposures to carcinogens and minimizing the role of occupational carcinogens. These considerations further illustrate the primary thesis of *The Politics of Cancer*: cancer is essentially a preventable disease which requires intervention and regulation at several levels, particularly the occupational and smoking. Failure to prevent cancer reflects major political and economic constraints which have hitherto been largely unrecognized or discounted.

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ARTICLES

Proterozoic transcurrent tectonics: palaeomagnetic evidence from Venezuela and Africa

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Comparison of palaeomagnetic poles from the Guayana Shield with the published Precambrian palaeomagnetic record for Africa suggests that large-scale (thousands of kilometres) transcurrent motions have occurred along Upper Proterozoic mobile belts in South America and Africa. Displacements of such magnitude are consistent with a transform model for Precambrian mobile belts.

DISCUSSION of the role of plate tectonics in Precambrian crustal evolution has focused on the significance of the mobile belts that partition Precambrian shields (see Fig. 1). They could have been formed by intracratonic vertical movements and crustal thinning induced by mantle upwelling along hotspot tracks or ridge systems^{1,2} (ridge-lineament model), or they may be the scars of the subduction zones along which the two adjacent cratons were 'welded' together³ (suture model). The ridge-lineament (suture) model implies small (large) relative motions among cratonic nuclei.

It is also possible that Precambrian mobile belts are zones of transcurrent movement^{4,5}. This hypothesis would predict differential motions of a different type among adjacent cratons. The suture model requires the strike of the mobile belt to fall approximately along a meridian (longitude) passing through the relative rotation pole of the adjacent cratons; whereas in the transcurrent model, the strike of the mobile belt will tend to lie along a latitude line with respect to the relative rotation pole.

With a sufficient amount of reliably dated palaeomagnetic data of the same age from each of the bordering cratons, it is theoretically possible to determine which of the three models best fits the data. We compare here newly obtained Precambrian palaeomagnetic data from the northern part of the Guayana Shield with the published Precambrian palaeomagnetic record for the West African, the Kalahari and the Congo shields. Although the palaeomagnetic data base is still rather small, we believe the data are more consistent with the transcurrent model than the other two models. Our results, however, as in other published intercratonic comparisons of Precambrian palaeomagnetic data⁶⁻¹¹, are not completely unambiguous.

Our palaeomagnetic data (Table 1) are based on a study of 300 samples (47 sites) from five major stratigraphic units of the Venezuelan Guayana Shield: the granulites of the Imataca Complex (2,000–2,050 Myr)^{12,13}, the Supamo–Pastora granite–greenstone terrain (1,900–2,000 Myr)¹⁴⁻¹⁶, the Cuchivero–Caicara acid metavolcanics (1,600–1,900 Myr)¹⁷⁻²⁰, the Roraima sandstone and diabases (1,660)²¹⁻²³ and the Parguaza rapakivi batholith (1,520–1,590 Myr)¹⁷.

Results of the analysis

Two or more components of magnetization were isolated by alternating field demagnetization from most of the sites. Thermal demagnetization of pilot samples of the Imataca Complex and the Roraima Group proved less effective than alternating field demagnetization in separating magnetic components. Examples of alternating field and thermal demagnetization results are given in Fig. 2. In general, the mean site directions of

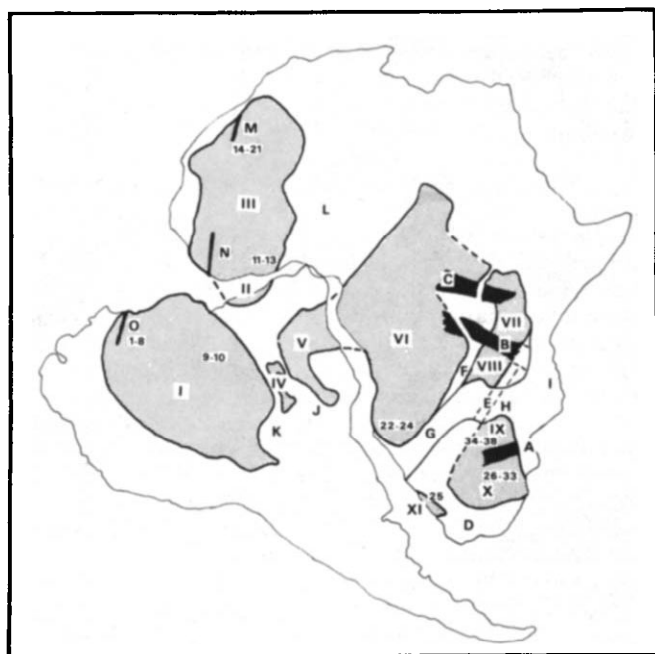


Fig. 1 Stable continental crust since 1,500 Myr BP, and mobile belts in Africa and South America. Plot is on the Bullard *et al.*³⁶ reconstruction. Stippled areas represent continental crust stable since 1,500 Myr. I, the Guayana Shield. The West African Shield is composed of the West African Craton (III) and the Sao Luis Craton (II). The Congo Shield is composed of the Sao Francisco Craton (V), the Kasai Craton (VI), the Tanzanian Craton (VII) and the Zambian Craton (VIII). The Kalahari Shield is composed of the Rhodesian Craton (IX) and the Kaapvaal Craton (X). XI is the Richtersveld Province, and IV is the Goias Central Massif. The Lower Proterozoic age (2,000 Myr.) mobile belts comprise the Limpopo belt (A), the Ubendian–Rusizian belt (B) and the Kibali–Toro–Bugandian belt (C). The Kibaran age (1,100 Myr) belts consist of the Namaqualand belt (D), the Irumide belt (E) and the Kibari belt (F). The Pan African age (600 Myr) belts comprise the Damara belt (G), the Zambezi belt (H), the Mozambique belt (I), the Brasilia (Brazilian) belt (J), the Paraguay–Araguaia belt (K) and the Dahomeyan–Pharusian belt (L). M is the Sfarit zone, N is the Sassandra mylonite zone and O is the Guri mylonite zone. Arabic numerals refer to the pole numbers in the first column of Table 1.

the more stable component as given by minimum dispersion analysis²⁴ were not significantly different from those given by vector analysis of Zijdeveld diagrams^{25,26}. The poles reported in Table 1 are averages of site poles based on at least four samples and with values for Fisher's $k > 7$. The mean site poles for the Imataca Complex and the Roraima Intrusive Suite were

Table 1 Average palaeomagnetic poles for South America and Africa 1,500–2,200 Myr

Location-formation	Age (Myr)	No. of sites	Pole lat (N°)	long (E°)	k	A95	Ref.
Guayana Shield							
<i>Venezuela</i>							
1. Supamo–Pastora*	2,300–2,250‡ 1,900–2,000§	2	–50	346†	9	98	This work, 14–16
Imataca							
2. Pole I	2,055–2,000	9	–49	18†	34	9	This work, 12, 13
3. Pole II	2,055–2,000	4	–29	21	48	13	This work, 12, 13
4. Cuchivero–Caicara	1,960–1,640	6	–69	70	10	22	This work, 17–20
5. Roraima sandstone	1,660	3	–69	347†	27	24	This work, 21
Roraima Intrusive Suite							
6. Pole I	1,660	9	–61	41	50	7	This work, 22, 23
7. Pole II		9	–44	353	54	7	60–62
8. Parguaza	1,590–1,524‡§	2	–67	26	407	13	This work, 17
<i>Brazil</i>							
9. Telles Pires	1,610	18¶	–69	292	—	9	39
10. Beneficente Gp.	1,480	21¶	–75	31	—	5	39
West African Shield							
<i>Ghana</i>							
11. Obuasi greenstone	2,150–1,670§	5	–49	99	23	16	32, 33, 40
12. Obuasi dykes	2,150–1,670§	14¶	–56	69	22	—	32, 33, 40
13. Tarkwa dykes	2,150–1,670§	5	–53	36	32	14	32, 33, 40
<i>Algeria</i>							
14. Aftout gabbro	1,940	4	29	53	185	7	28, 31
15. Aftout gabbro	1,940	2	–6	97	8	>90	28, 31
16. Aftout diorite	1,940	3	38	45	45	19	28, 31
17. Aftout diorite	1,940	3	–20	80	65	15	28, 31
18. Aftout diorite	1,940	4	58	73	89	13	28, 31
19. Akilet dykes	<2,160	5	28	81	32	14	28, 31
20. Akilet dykes	<2,160	3	–16	54	52	17	28, 31
21. Akilet dykes	<2,160	13	–16	95	17	10	28, 31
Sahara–Congo Shield							
<i>Angola</i>							
22. Cunene anorthosite	1,870–2,160	2	–26	16	18	63	29, 47
23. Cunene anorthosite	1,870–2,160	3	28	55	17	31	29, 47
24. Cunene anorthosite	1,870–2,160	6	–3	92	29	13	29, 47
Richtersveld Province							
<i>Namibia</i>							
25. Orange River Lavas	2,000	5	–5	93	5	40	40, 42
Kalahari Shield							
<i>South Africa</i>							
26. Lower. Ventersdorp Lavas	2,150	24¶	–3	42	11	9	43
27. Bushveld Complex	2,050	5	23	36	40	12	41, 44
28. Vredefort Complex	1,900	8	26	39	21	12	63, 64
29. Losberg Intrusion	1,840	3¶	33	37	182	9	
30. Palabora Complex	2,060‡	6	41	48	61	7	65
31. Palabora Complex		4	3	359	119	8	
32. Waterberg red beds	<1,790‡	4	2	334	29	17	66, 67
33. Waterberg red beds	<1,790‡	4	43	45	47	11	66, 67
<i>Zimbabwe</i>							
34. Great Dyke extensions	2,000	2	6	47	1,413	3	68, 69
35. Great Dyke extensions	2,000	2	–2	333	248	16	68, 69
36. Great Dyke extensions	2,000	11	29	4	45	7	68, 69
37. Mashonaland dolerites	1,810	14	13	341	51	6	45, 46

* Details on our site locations and on our palaeomagnetic data will be presented elsewhere²⁷.

† The palaeomagnetic data for the Imataca Complex, the Supamo–Pastora province and the Roraima Group have not been structurally corrected. The Imataca Complex fails the fold test at the 95% level of confidence. Although the between-site *k* values improve after structural corrections for the Supamo–Pastora data (from 6 to 12) and for the Roraima Group data (from 12 to 15), these are not significant improvements at the 95% confidence level. For the Roraima dykes, the poles given here are the average of the data obtained in this study and previously published results^{60–62}.

‡ U–Pb ages.

§ K–Ar ages.

|| Rb–Sr ages, in many cases whole-rock isochrons, $\lambda^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$.

¶ Number represents total number of samples averaged.

distributed in clusters (separated by at least 120°). The mean and associated fisherian statistics for each cluster are reported (Table 1).

Although the protolith age¹² for the rock units of the Imataca Complex is >3,000 Myr, their stable remanence was probably acquired during regional uplift and cooling following granulite metamorphism, which has been dated at 2,050–2,000 Myr by a Rb–Sr biotite age¹³ and detailed Rb–Sr and U–Pb analyses of the granulites¹².

The stable remanence in the Supamo–Pastora rock units was also probably acquired during regional uplift and cooling, which

has been dated at 2,000–1,900 Myr based on K–Ar hornblende and biotite ages¹⁵.

The origin of the stable remanence in the Cuchivero–Caicara metavolcanics is uncertain; however, because of their low metamorphic grade (lower greenschist) and lack of multiple components of magnetization, the age of their remanence is probably close to their Rb–Sr whole-rock isochron ages^{17–20}, which range from 1,960–1,640 Myr. The consistency in the remanence directions from sites separated by >400 km suggest little structural deformation has occurred since the acquisition of their magnetization.

Among the data from the Imataca Complex, the within-site concentration as given by Fisher's k varied widely from 6 to 255; the between-site concentrations were fairly high (Table 1). The within-site k values for the Supamo-Pastora and Caicara-Cuchivero units ranged from 10 to 165.

The more stable components present in the younger intrusive units, the Roraima diabbases and the Parguaza batholith, are probably primarily thermoremanent magnetizations acquired at 1,660 and 1,500 Myr. The within-site k values for the Roraima diabbases were quite high, ranging from 17 to 230; those for the Parguaza were variable, ranging from 3 to 62.

The age and nature of the remanence of the Roraima sandstone are uncertain because hydrothermal alteration is pervasive and the remanence could easily be a chemical overprint.

Many of the less stable secondary directions observed in both the metamorphic and intrusive units represent partial remagnetization during the low-grade Nickorie metamorphic episode¹⁵ dated at 1,200 Myr. Eight of the sites were apparently partially remagnetized during this metamorphic episode. These

secondary overprint directions have not been included in Table 1, but will be discussed elsewhere²⁷.

Palaeomagnetic data

The Precambrian palaeomagnetic poles from the three major African cratons given in Table 1 have been recalculated from the original site-pole distributions. In most cases, the pole obtained is the same as that reported in the original publication. In the data from studies in Algeria²⁸, Angola²⁹ and Namibia³⁰, however, the site-pole distribution showed several distinct clusters. We have calculated poles for each of these clusters rather than a single site-mean pole as originally reported. We believe that the several clusters may be records of the geomagnetic field at different times, and that information is lost by calculating only one mean pole position. Our conclusions, however, do not depend on this operation, and the original published poles could equally well have been used.

All of the African palaeomagnetic data reported in Table 1 and Figs 3, 4 and 5 are based on at least 10 independent samples and have a Fisher's $k \geq 5$ (only three poles have $k < 10$).

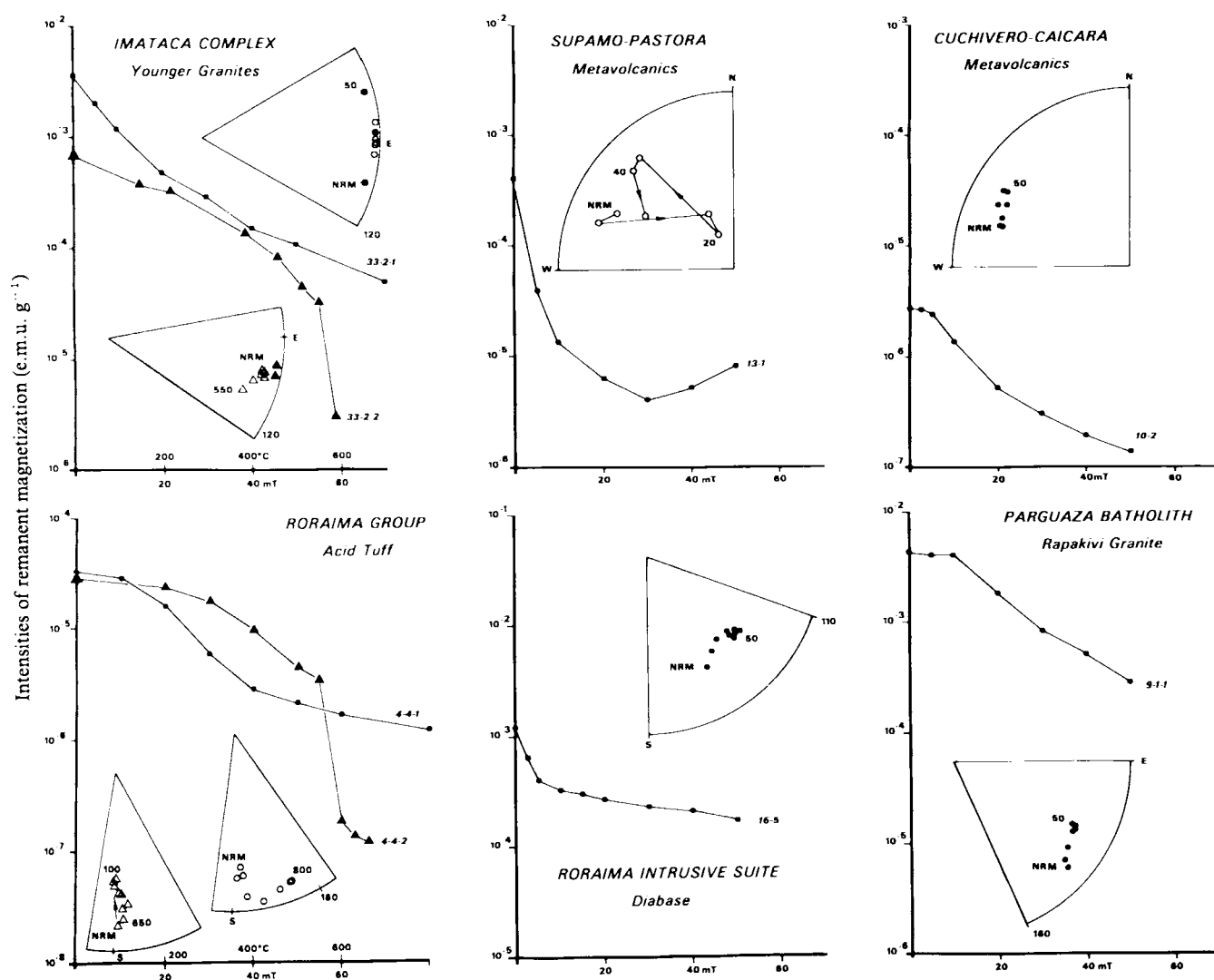


Fig. 2 Progressive demagnetization studies. Representative examples of thermal (▲) and alternating field (●) demagnetization of pairs of specimens from the same oriented samples of the Imataca Complex and the Roraima group, and representative examples of alternating field (●) demagnetization for specimens of the Pastora metavolcanics, Cuchivero metavolcanics, the Roraima diabase and the Parguaza batholith. The directions of remanent magnetization are plotted on equal-area projections, the plane is the present horizontal. Amphibolites and granites intrusive into the Imataca Complex tend to have very stable single-component magnetizations; whereas, mafic granulites, charnockites and biotite gneisses tend to have multicomponent magnetizations. Blocking temperatures indicate that magnetite is the principal carrier of the remanence; in polished sections magnetite is frequently present as very fine rods in pyroxene grains. The remanent magnetization of the greenschist facies rock units of the Pastora tends to be very weak and migrate widely on demagnetization. The remanent magnetization of the very low-grade Cuchivero metavolcanics and the tuffs of the Roraima group tends to be weak, but extremely stable. Blocking temperatures suggest that the magnetization is primarily carried by magnetite, which is present in the groundmass as very fine equant grains. The remanent directions tend to migrate rapidly to a stable, characteristic direction with alternating field demagnetization. The secondary directions seem to be lightning induced. Samples of the Parguaza rapakivi granite exhibited a wide range of demagnetization behaviour, from extremely high coercive single-component magnetization to low coercive multicomponent magnetizations.

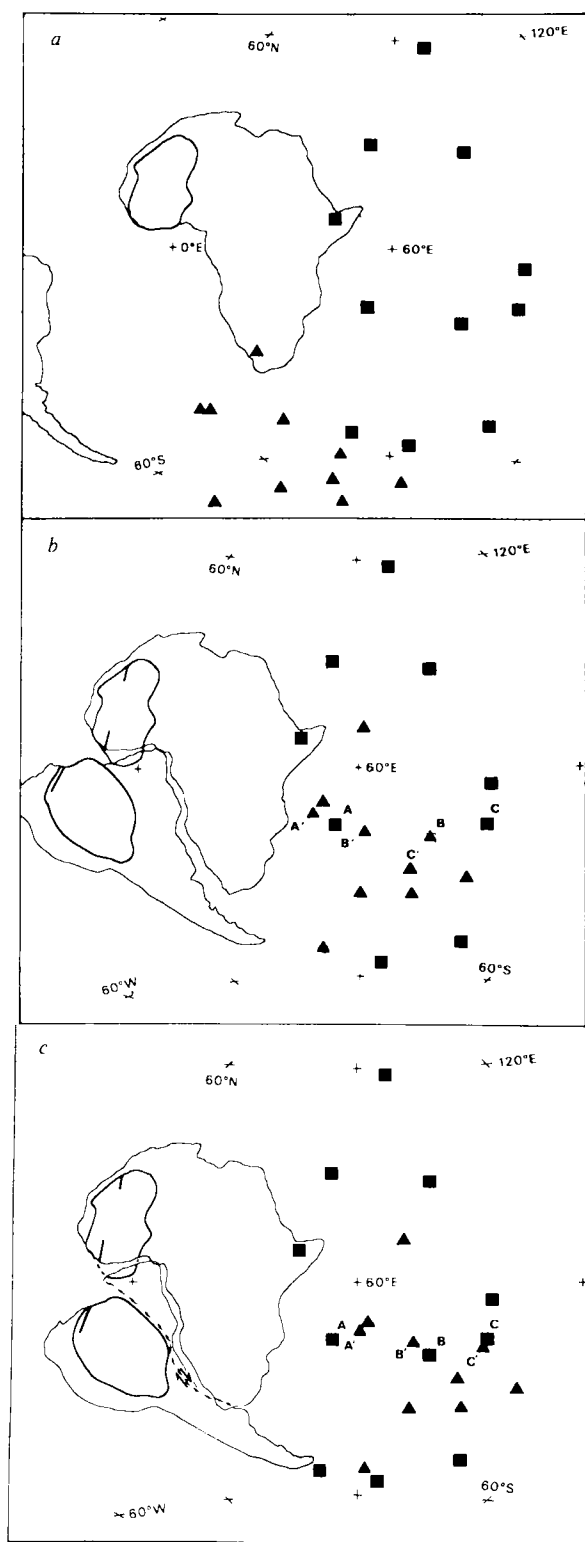


Fig. 3 Palaeomagnetic data from the South American Guayana Shield ($\blacktriangle$) and the West African Shield ($\blacksquare$) for 1,500–2,100 Myr. Continental crust older than 1,500 Myr and the Guri and Sassandra fault zones are shown (thick lines). *a*, Continents in their present day position. *b*, Reconstruction according to ref. 36, with A, B and C and A', B' and C' indicating the more reliably dated poles from the West African Shield and the Guayana Shield, respectively. *c*, Proposed reconstruction for the period 2,100–1,500 Myr, which brings together the correlatable data points from each shield and also aligns the basement structures between the two shields. Transcurrent fault is shown with direction of displacement indicated by small arrows.

Radiometric ages given in Table 1 are based on data given in refs 15, 16.

The West African data are from a sequence of rock units geologically similar to those in the Guayana shield: a metamorphic basement (1,800–2,000 Myr)^{31–33} with intrusive diabases (1,300–1,700 Myr)^{28,34}. Comparison of Fig. 2*a* and *b* shows that the South American data (triangles) do agree much better with the West African data (squares) after the South Atlantic has been closed using a Bullard *et al.*³⁶ fit, in agreement with the hypothesis of the Hurley and Rand³⁵.

Detailed comparison of one of the Imataca granulites and both of the Roraima diabase poles (numbers 3, 6 and 7 marked A', B' and C' in Fig. 3*b, c*) with the most likely correlative poles from the West African Shield, the Aftout diorite pole and the Akilet dyke poles (numbers 17, 20 and 21 marked A, B and C in Fig. 3*b, c*) suggests a slight westward offset in the South American data. This offset could be eliminated by rotating the Guayana Shield (Fig. 3*c*) 13° counterclockwise about a pole 70°E, 66°N. Such a rotation would require ~1,500 km displacement along a fault zone postulated to lie in the Brazilian mobile belt (Fig. 1). After this shift, the Sassandra mylonite zone³⁷ (Fig. 1), which separates the granulite rock units of Ivory Coast from the Birrimian greenstones, becomes continuous with the Guri mylonite zone which separates the Imataca granulites from the Pastora greenstones³⁸ (Fig. 3*c*). This alignment suggests that the proposed translations may be correct.

In comparing the data from the Guayana Shield and the West African Shield with those from the Congo Shield and the Kalahari Shield (Figs 4, 5), we have shown only those data that fall in the 1,800–2,100-Myr age range, because no palaeomagnetic data in the 1,500–1,800-Myr age range exist for the latter two shields. In Fig. 4 the three poles from Ghana (11–13 in Table 1, Fig. 1) are significantly removed from other contemporaneous West African poles as well as from the poles of the other shields. As observed in Fig. 3*b, c*, the Ghana poles do coincide with a pole from Brazil, for which ages of 1,500–1,600 Myr have been reported³⁹. This correlation leads us to suspect that the age of the magnetization for the poles from Ghana may be younger than the 2,200-Myr age given for them⁴⁰. For this reason and to clarify the diagram, all the Ghana poles have been omitted from Fig. 5.

The principal observation from Fig. 4 is that the palaeomagnetic data from the Kalahari Shield fall mainly in eastern and northern Africa. In contrast the palaeomagnetic poles from all the other cratons are concentrated in the Indian Ocean. The most recent age determinations^{12,41,42} for both data sets strongly suggest that this discrepancy cannot be the result of differences in age.

The Kalahari palaeomagnetic poles exhibit a consistent succession in age from the Lower Ventersdorp Lavas pole (number 26, 2,150 Myr)⁴³, through the Bushveld Complex pole (number 27, 2,000 Myr)^{41,44}, to the Mashonaland dolerites pole (number 33, 1,800 Myr)^{45,46}. The data from the Cunene Anorthosite Complex (numbers 22–24, 1,800–2,100 Myr)^{29,47}; diamonds in Figs 4 and 5) of the Congo craton and from the Orange River Lavas (number 25, 2,000 Myr)^{30,42}; solid hexagon) of the recently recognized Richtersveld Province in Namibia⁴⁸ (Fig. 1) are distinct from those of the Kalahari, and compare favourably with poles from the West African and Guayana shields; a temporal distribution pattern for these latter poles is roughly comparable with that displayed by the Kalahari poles (Fig. 4). The best superposition of these two apparent polar wander curves as drawn is obtained by rotating the Guayana, West African and Congo shields and the Richtersveld Province 64° counterclockwise about a pole at 44°E, 28°S (Fig. 5).

As the Richtersveld Province (XI in Fig. 1) also takes part in the rotation, a corresponding zone of right lateral transcurrent displacement must separate it from the Kalahari Shield (IX and X in Fig. 1). The only possible location for this zone is in the 1,100-Myr old Namaqualand mobile belt (D in Fig. 1). Similarly, the Kasai craton (VI in Fig. 1) must be separated from

the Kalahari Shield by the northward continuation of this zone of dislocation, which would presumably lie within either the Irumide or the Kibaran mobile belt (E and F, respectively, in Fig. 1), as they are equivalent in age to the Namaqualand belt. If the rotation is attributed solely to transcurrent motion, then a small circle about the rotation pole (P in Fig. 5), passing through the Namaqualand belt, coincides more closely with the Irumide belt than with the Kibaran belt. The rotation would require ~2,000 km of right-lateral displacement within this combined Namaqualand–Irumide mobile belt. As no palaeomagnetic data exist for the Zambian–Tanzanian cratons for this time period, we cannot preclude the possibility that they remained fixed with respect to the Kalahari Shield, and that the displacement occurred along the Kibaran belt. Note, however, that after our postulated rotation, along the Irumide belt, the Limpopo belt (A in Fig. 1) and the Ubendian–Rusizian belt (B in Fig. 1) become continuous (Fig. 5). We consider this result to support primary displacement occurring along the Irumide belt.

Discussion

The geology of Southern Africa is generally consistent with our hypothesis except in the critical area south-east of the Zambian craton where it is extremely complex and much disputed⁷⁰. Ramsay and Ridgway⁴⁹ find no evidence for an Irumide belt, and propose that the Pan African Mozambique belt forms the eastern margin of the Zambian (and Tanzanian) cratons. They do admit, however, that older gneisses of Ubendian or Kibaran (Irumide) age may be present within the Mozambique belt. Shackleton⁵⁰ recognizes an Irumide belt, but proposes that it terminates south-west of the Ubendian belt, which he concludes can be connected to the eastern end of the Limpopo belt through a trend of granulites in the Mozambique belt. In addition, Key and Sutton⁵¹ propose that the Limpopo belt does not continue westwards completely through the Kalahari shield (Fig. 1), implying that its alignment with the Ubendian belt after rotation (Fig. 5) is fortuitous. Nevertheless, the palaeomagnetic evidence for our hypothesis is compelling, and hopefully any apparent inconsistency with the geology may eventually be resolved.

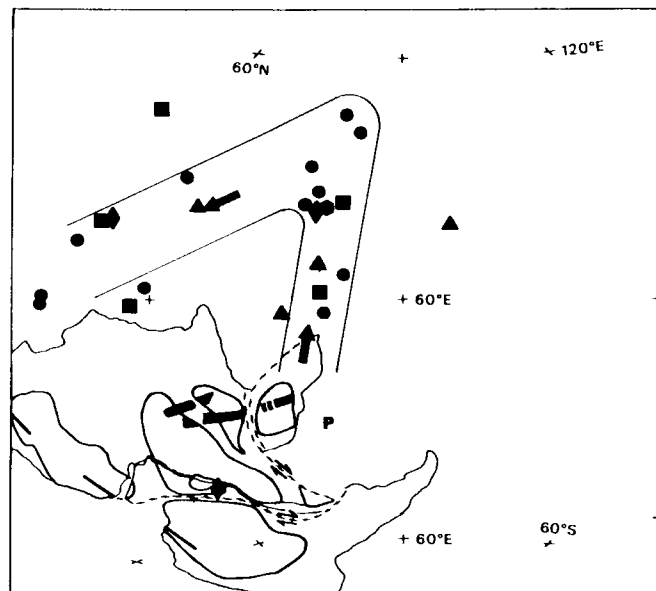


Fig. 5 Palaeomagnetic data same as in Fig. 4, except the data from Ghana have not been included. Continents are in proposed reconstruction, which brings together the two APW paths and aligns the Limpopo belt and the Ubendian–Rusizian belt. P is the pole of rotation, and the transform fault zone is shown with displacement indicated by small arrows. See Fig. 4 for remaining symbols.

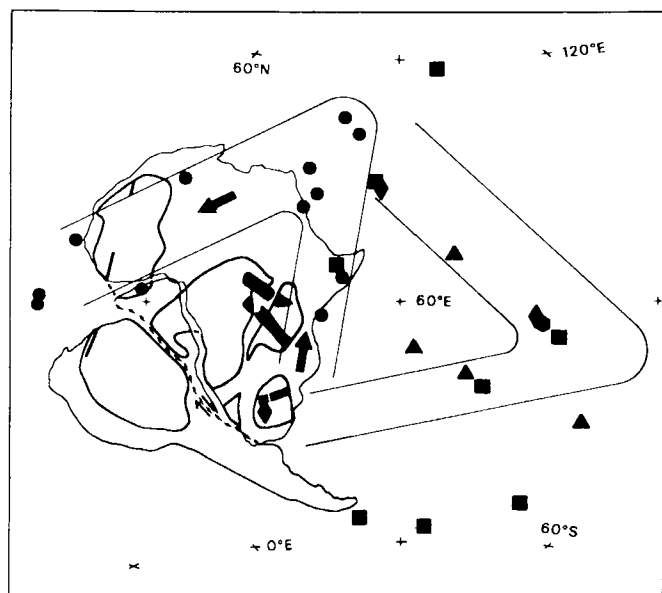


Fig. 4 Palaeomagnetic data for the Kalahari Shield (●), the Congo Shield (◆), the Orange River Lavas (◐), the West African Shield (■) and the Venezuelan Guayana Shield (▲) for 1,800–2,100 Myr. Suggested apparent polar wander (APW) path for the Kalahari data is outlined and the direction of polar wander indicated (black arrows). Proposed correlative APW for the data from the other shields is outlined. The three solid squares near the bottom of the figure are the data from Ghana⁴⁰ and have not been included in the proposed APW path (see text). Continental crust older than 1,500 Myr (enclosed by thick lines), correlative fault zones (thick lines) and correlative mobile belts (solid) are shown. Continents are in the same reconstruction as Fig. 3c.

Large-scale (thousands of kilometres) transcurrent displacements along Phanerozoic orogenic belts have been documented palaeomagnetically and geologically^{52–55}. The above palaeomagnetic analyses suggest that large-scale transcurrent motions have also occurred in Proterozoic mobile belts, particularly those for which intense shearing and smaller-scale transcurrent displacements have been reported^{56–59}. Without proper corrections for such movements polar wander paths based on an expanding intercratonic palaeomagnetic data base either will continue to become more complicated and convoluted, or the path widths and the apparent uncertainties in the ages of magnetization will be enlarged when attempts are made to keep the polar wander paths relatively simple.

If palaeomagnetic evidence is found for similar intercratonic movements in other continents then the transcurrent-tectonic model advocated here may signify a tectonic style in the Precambrian similar to that proposed by Sutton and Watson¹⁰, which was characterized more by transcurrent shearing (jostling) of cratonic blocks than by the rifting, spreading and closing of oceans.

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The *b*-value as an earthquake precursor

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A systematic study of b-values in New Zealand has shown that within the vicinity of forthcoming large earthquakes there is initially an increase in b and then a return to normal. The Caracas and San Fernando earthquakes show the same phenomenon, which is related to that of seismic gaps.

IN the recurrence relation of Gutenberg and Richter¹, $\log N = a - bM$, the parameter *b* measures the relative numbers of small and large earthquakes. Values near unity are common. The value of *b* is usually calculated by the method of maximum likelihood (see ref. 2) whence

$$b = \frac{\log_{10} e}{\bar{M} - M_0}$$

$\bar{M}$ is the mean magnitude of the sample and M_0 the minimum magnitude considered. The standard error in *b* is $b/\sqrt{n}$ for a sample of *n* earthquakes² and the 95% confidence limits are twice this value.

Fiedler³ found that *b*-values increased before the Caracas earthquake of 1967. His estimates were of the 'extreme *b*-value' for each year, and they increased to 1.33 before dropping again before the earthquake. Wyss and Lee⁴ considered a sample of many more earthquakes, of much smaller magnitudes, in California. They calculated *b* using the maximum likelihood method and a 50-event window, and claim that it decreased both before and after main events. In their data the decrease before the main events took place over some hundreds of days. But there is also a suggestion of an increase in *b*, followed by a decrease just before the main event, the changes taking place over a few tens of days (for example at Danville, late 1971, where the magnitude of the main shock was 3.4).

Studies of aftershock sequences in New Zealand have also found that the *b*-value is not constant in time. In some cases Gibowicz⁵ found an increase after the main event, followed by a decrease before the next large shock. He also found large changes in *b* for large areas of the North Island⁶, as did Robinson⁷, who found an increase to a peak value of 1.75 in 1971, followed by a decrease.

This paper examines further evidence relevant to Fiedler's suggestion³ that a period of high *b*-values can occur before an earthquake.

High *b*-values in small regions

A systematic study of the temporal variation of *b*-values throughout New Zealand has now been made, using regions 2° in latitude by 2° in longitude, earthquakes of magnitude 4.0 and greater from 1955 to June 1979, focal depths less than 40 km, and a 50-event window which slides by 10 events. Smaller regions did not contain enough data for adequate resolution of the *b*-values. No attempt has been made to remove swarms and aftershocks from the data. Results for several of the regions are shown in Fig. 1, where the *b*-value is plotted at the time the last event entered the window. All five plots in Fig. 1 begin with low values of *b*, and this implies a lack of detection of small events before the mid 1960s when the New Zealand seismograph network was expanded to reduce the detection threshold to

Table 1 Total precursor times, from the onset of the increase in *b*-values to the occurrence of the earthquake

Earthquake	Magnitude	Precursor time
Tejon Pass, 1961	5.0	3.5 yr
Caracas, 1967	6.7	7 yr
Inangahua, 1968	6.8	6 yr
San Fernando, 1971	6.4	7 yr
Region E, 1971	5.9	3.2 yr
Region C, 1973	5.7	4 yr
Region A, 1977	6.0	7 yr
Pukaki, 1978	3.8	6 months
Pukaki, 1978	4.6	16 months

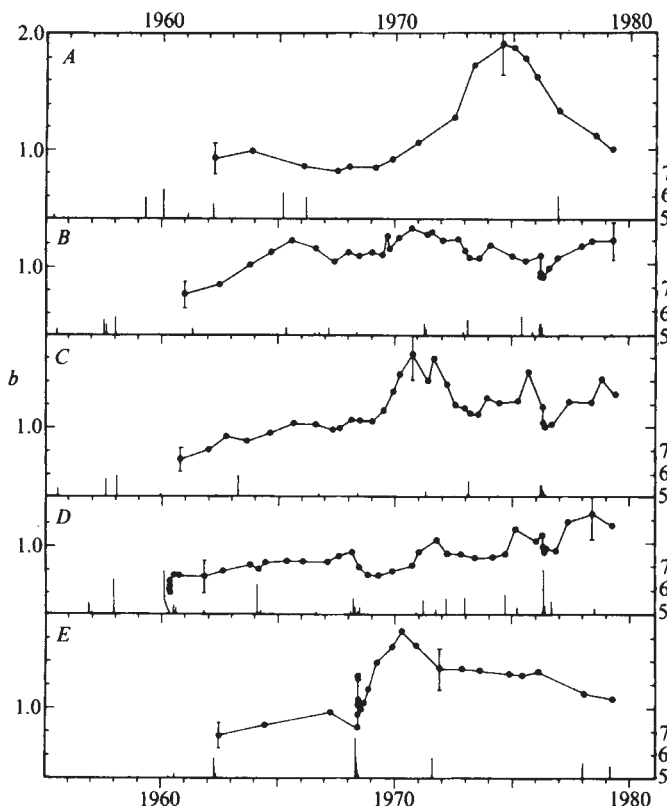


Fig. 1 The variation of parameter b from 1955 to 1979, in each of five regions of New Zealand. A location map is given in Fig. 2. Standard errors are proportional to b and are shown in a few cases, as are earthquakes of magnitude greater than 5. A 50-event window has been used, sliding by 10 events.

magnitude 4.0 throughout the country. Standard errors, proportional to the ordinate because of the uniform window length, are shown in several cases. Also shown are the earthquakes of magnitude >5 in each area. A location map is shown in Fig. 2.

In region A the b -value rose to a peak of 1.9 in 1974 and then decreased. During the decrease there was an earthquake of magnitude 6.0. The high b -values were accompanied by a paucity of moderate earthquakes. During the period 1969–76, while the b -value rose to its peak then began to drop, there were no earthquakes over magnitude 5.0 in the region.

Figure 1B shows the b -value variation for another area, in the east of the North Island. Little variation is evident, although there was an earthquake of magnitude 5.9 in June 1975. Figure 1C plots a region in the north-east, where a high value of b (1.6) was followed by an earthquake of magnitude 5.7 in 1973 which occurred near the city of Napier. Figure 1D shows a similar plot for the Fiordland area, exhibiting very little variation in b in spite of the large earthquake in 1976. This point is taken up again later.

Figure 1E shows the Inangahua earthquake of 1968 (magnitude 6.8), and a marked increase in b which followed it. It is clear that high values of b , while they may be associated with imminent earthquakes, may also relate to previous activity. If Fig. 1E is any indication, however, the previous event should be easily identified, and in this case the period of high b -values could also be associated with the magnitude 5.9 event in 1971.

Figure 3 shows data obtained from the Lake Pukaki micro-earthquake network, situated in the South Island of New Zealand near 44°S, 170°E. This network of nine stations was installed in 1975 to monitor seismicity there during the raising of the lake level for hydroelectricity generation. b -Values have been calculated for magnitudes 1.5 and greater, using a 50-event window which slides by 10 events. In December 1978 an earthquake of magnitude 4.6 occurred close to the centre of the

network⁸. This event was preceded by a period of high b -values. There may also be another occurrence of the same phenomenon before the magnitude 3.8 shock of August 1978, superimposed on the larger trend.

Further evidence concerning whether or not periods of high b -value are followed by large earthquakes must be sought in other seismic regions, by systematic studies such as has been done in New Zealand. But the converse—whether or not large earthquakes are preceded by periods of high b -value—is examined in Fig. 4. The region 34–35°N, 118–119°W includes the San Fernando earthquake of 1971. Data selected from the Southern California catalogue⁹ are shown in Fig. 4A. The magnitude threshold is 2.5 and the window length is 20 events, sliding by five events. Decreasing the window length was necessary because of the small area chosen and the generally low level of seismicity, even at the magnitude 2.5 level, but, of course, the standard error increases. Two periods of high b -value are evident (approximately double the background level), one before the earthquakes of 1961 and 1963 in the Tejon Pass quadrangle, and the other before the San Fernando earthquake of 1971. The data set did not include the San Fernando earthquake itself.

Figure 4B shows a reexamination of the data for area e (see Figs 1 and 2). The window length has been reduced to 20 events, sliding by five events. The b -value increased to 2.2 before the Inangahua earthquake of 1968. This is an alternative expression of the precursory gap which Evison¹⁰ found for that earthquake. The area is of only moderate seismicity, and detection may not have been complete to magnitude 4.0 in the early 1960s. Both of these reasons demanded the short window. There is no clear decrease in b before the earthquake, but note that the final value of 2.2 at the beginning of 1968 represents an average back to 1963. There is clearly insufficient data to resolve any decrease which may have occurred before the major event. Extending the data sets to include the San Fernando and Inangahua earthquakes returns the b -value to its background level in each case.

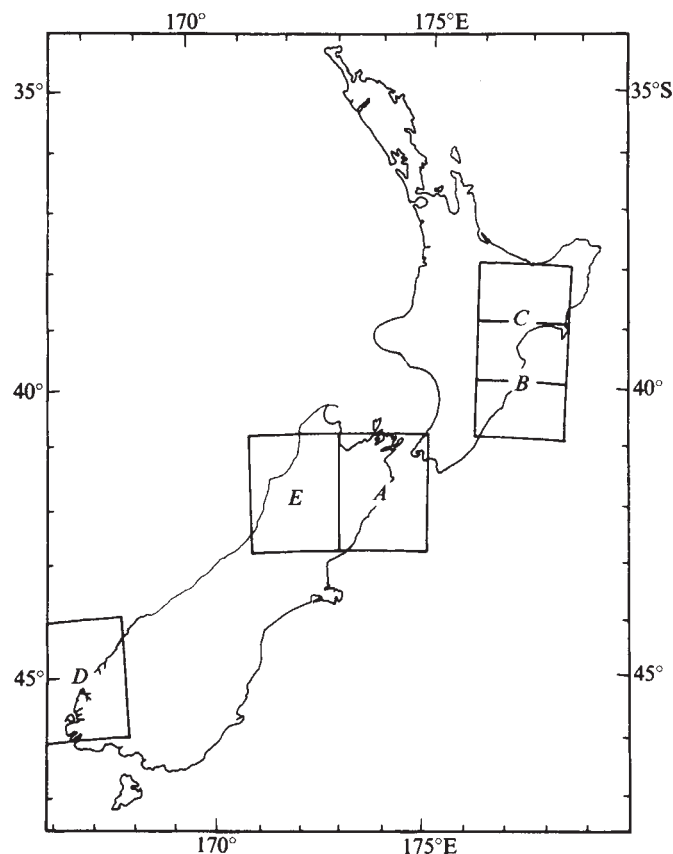


Fig. 2 Location map for the regions of Fig. 1.

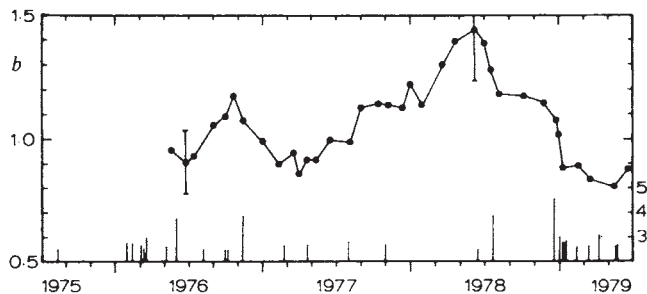


Fig. 3 b -Values for the Lake Pukaki seismic network. Magnitude threshold 1.5, window length 50 events, sliding by 10 events. Earthquakes of magnitude 3.5 and greater are also shown.

The Fiordland area (Fig. 1D) is very active, and if there are precursory changes in b for the large events shown, it will be necessary to decrease the search area to isolate them. Precursory changes for individual earthquakes, if they occur, are obscured by the high level of activity. The same problem is also likely to occur in Japan or any highly active area. It may be intensified in Fiordland where the source regions of earthquakes are generally small.

It is interesting to simulate these windowed b -value calculations using a synthetic data set with an exponential frequency distribution. The b -value so calculated does, of course, fluctuate. In fact the probability that the observed changes in b with real data occurred by chance is significant (if the earthquake population does actually have a random exponential distribution), but this is not the point. The proper question is: 'Are periods of high b -value followed by large earthquakes?'. The evidence presented here suggests that they are.

Note also that b -values have been calculated only as a statistical parameter of the small samples. No extrapolation to large samples is implied. The b -value is directly related to the mean magnitude, which may be a more useful parameter, but the terminology has been retained in favour of the well-known parameter b . A high b -value implies a low mean magnitude.

The b -value as an earthquake predictor

The evidence presented, that a period of high b -values is followed by a large event, is alternately expressed in the gap hypothesis. Mogi's¹¹ "seismic gap of the second kind", defined

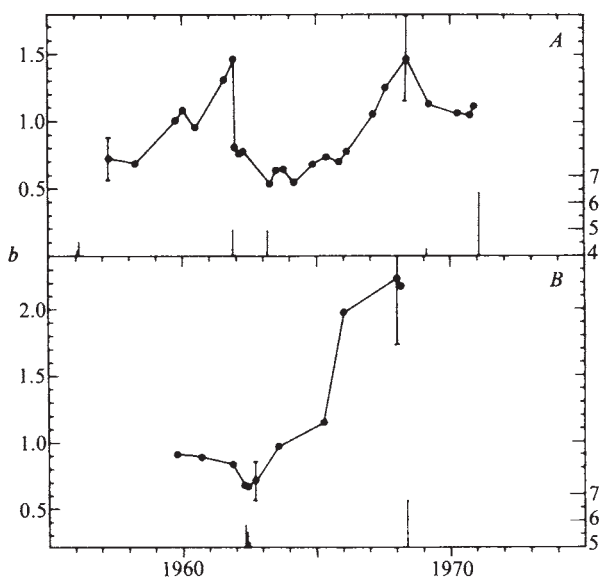


Fig. 4 b -Values for: A, the region of the San Fernando earthquake of 1971 (34–35°N, 118–119°W; $M \geq 2.5$) and B, the Inangahua earthquake of 1968 (41–43°S, 171–173°E; $M \geq 4.0$). Window length 20 events, sliding by 5 events. Earthquakes of magnitude greater than 4.0 (A) and 5.0 (B) are also shown.

as a region deficient in moderate earthquakes before the occurrence of a large earthquake, may find expression in a high b -value. Evison's¹² suggestion of a precursory swarm followed by a gap may also be accommodated by identifying the swarm with the initial low value of b .

The b -values in each frame of Fig. 1 are calculated for an area rather larger than the precursory gap area for any of the earthquakes. This is encouraging for a surveillance scheme, if the b -value does indeed prove to be a predictor, because a routine procedure to examine the whole country for changes in b -value need not use search areas coincident with the aseismic area related to the forthcoming shocks it is endeavouring to detect. Provided that the search areas overlap the precursory area, but are not too much larger than it, the anomaly in b -values will be detected. The maximum b -value reached is not very significant, because of the mixed population it represents. What is apparently important is the temporal behaviour.

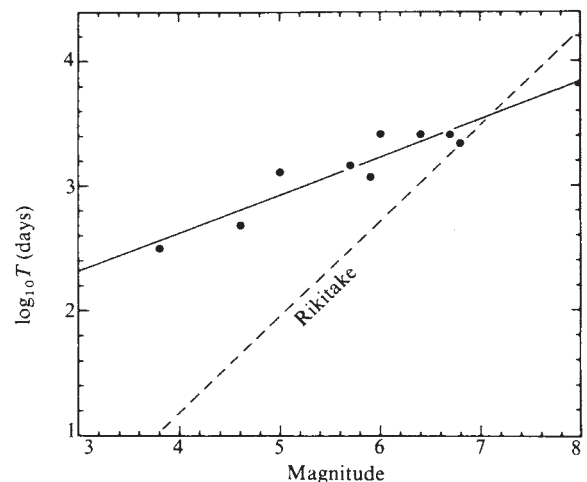


Fig. 5 Total precursor time T as a function of magnitude, for the earthquakes listed in Table 1.

Precursor time and magnitude

Total precursor times for the nine events identified are shown in Table 1, and in Fig. 5 as a function of magnitude, with the least squares regression:

$$\log T = 1.42 + 0.30M$$

For the Caracas event a magnitude of 6.7 has been used, as quoted by Rial¹³, although the ISC value of m_b was only 5.7. The regression is admittedly poor, and heavily dependent on the two Pukaki earthquakes of magnitude 3.8 and 4.6. But clearly the b -value does not fall into the class of phenomena which obey Rikitake's relationship¹⁴, also shown. The windowing technique can only reduce the observed precursor time, never increase it. This means that the ordinates plotted may be systematically small, except for the Caracas precursor which was determined from annual values. There seems to be no clear rule for the elapsed time from the b -value peak to the major earthquake, but this may be due to the windowing technique and the variety of seismicity levels in the data presented. The occurrence of foreshocks would tend to depress the b -value before a large event¹⁵, but this is a phenomenon on a much shorter time scale than that presented here.

Conclusions

The occurrence of a period of high b -values before a large earthquake has been observed in New Zealand, California and Venezuela. Only one case is known where it may have been associated with a previous event, the occasion when high b -values followed the Inangahua earthquake of 1968, but there was also a subsequent earthquake of magnitude 5.9 with which the high b -values could be associated. There may be a decrease

in *b* towards normal levels before the earthquake actually occurs, but it is difficult to resolve this in areas of low seismicity. The regression between magnitude and precursor time is poor, but the *b*-value clearly does not fall into the general class of phenomena which obey Rikitake's well known relationship.

I thank Drs E. G. C. Smith, R. Robinson and T. Hatherton for their helpful comments, and analysis staff at the Seismological

Observatory for painstakingly locating many thousands of small earthquakes. Dr Smith also performed the calculations on the synthetic data sets.

Note added in proof: Ma¹⁶ has also found evidence for high *b*-values before large earthquakes in China, including the Tangshan and Haicheng events.

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Actin-containing matrix associated with the plasma membrane of murine tumour and lymphoid cells

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A detergent-insoluble matrix has been isolated from murine tumour cell and lymphocyte plasma membranes. The major components of this matrix include actin and four additional proteins not previously identified as cytoskeletal proteins. Labelling studies indicate that the matrix is located on the inner face of the plasma membrane. A cell-surface glycoprotein, 5'-nucleotidase, remains associated with the isolated matrix.

THE cytoplasmic face of the plasma membrane appears to be the meeting place for two sets of cellular structures intimately involved in determining the response of a cell to its environment. It is here that the transmembrane cell-surface proteins may interact with components of the cytoskeletal system. The red cell membrane has served as an extremely useful model for understanding many aspects of the plasma membrane of more complex cells¹, but whether the similarity of red cell membranes and the plasma membranes of nucleated cells extends to one of the most striking features of the red cell membrane, the 'membrane skeleton', has remained unclear. Recent studies have revealed that the red cell membrane is not composed simply of a lipid bilayer with individual proteins interacting with it. Instead, spectrin and a group of associated proteins form an extensive network at the cytoplasmic face of the bilayer and function as a membrane skeleton to stabilize the membrane. This skeletal network controls the shape and elasticity of the membrane^{2,3} and the distribution of transmembrane glycoproteins⁴ and intramembrane particles⁵. The network can be readily isolated by extracting membranes with the non-ionic detergent Triton X-100. Spectrin, the major skeletal protein, along with the other network proteins and some of the membrane lipids, remains insoluble⁶. The results of numerous recent studies have led to an increasingly clear picture of how these proteins are organized to form the skeletal network⁷.

Spectrin is present in all mammalian and avian red cells but is not found in other cell types and a membrane skeleton similar to the spectrin network has not been demonstrated in nucleated cells. These cells have extensive filamentous cytoskeletal networks within the cytoplasm^{1,8,9} and it has been proposed that there are associations between transmembrane surface proteins and components of this cytoskeletal network at the inner surface of the membrane^{8,10}. These interactions may be involved in such phenomena as capping of surface molecules on lymphocytes^{11,12}, control of cell motility¹³ and attachment to surfaces¹⁴. Some direct evidence for interactions between transmembrane

proteins and components of the cytoskeletal system has been presented^{15–17}. Whether such interactions, primarily implicated in dynamic processes such as receptor redistribution or cell motility, might also serve to stabilize the membrane is unclear. It is also unclear whether a distinct structure exists on the cytoplasmic face of the membrane, analogous to the red cell membrane skeleton. The results reported here demonstrate that a detergent-insoluble component can be isolated from the plasma membranes of murine tumour and lymphoid cells which may function as a membrane skeletal network. The network is apparently located on the inner surface of the membrane and has five major protein components. Only one of these proteins, actin, has previously been shown to be a component of cytoskeletal structures.

Plasma membrane isolation and Nonidet P-40 solubilization

Plasma membranes were isolated as described previously for P815 ascites cells¹⁸ with only minor modifications (see Fig. 1 legend). This procedure results in a 40–50-fold purification, as assessed by 5'-nucleotidase activity, and a yield of 40–60%. Co-purification of the cell-surface H-2 antigens with the plasma membrane fraction (ref. 18 and unpublished data) confirmed the results obtained by assaying 5'-nucleotidase. Membranes have been purified from P815, EL4, RDM-4 and CH1 murine tumour cells and normal murine spleen cells with essentially identical results. Examination of the purified membranes by electron microscopy shows them to be in the form of vesicles of relatively heterogeneous size, ~0.1–1.2 µm in diameter¹⁸. Small amounts of endoplasmic reticulum are also apparent, as indicated by the presence of some membrane vesicles with bound ribosomes. Based on yields obtained, the plasma membrane accounts for 1–3% of the total cell protein, in good agreement with values reported by others¹⁹.

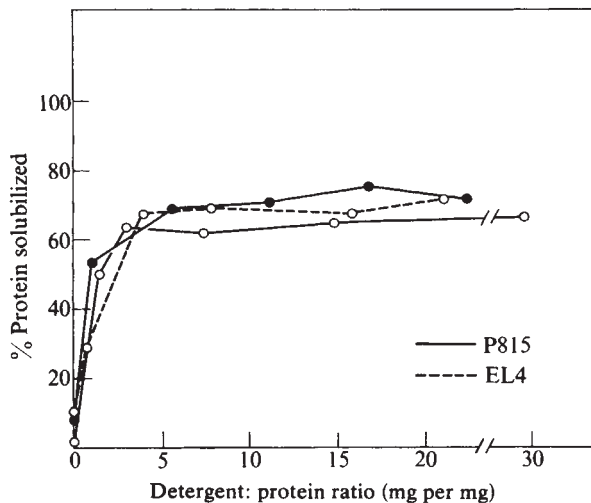


Fig. 1 Solubilization of P815 and EL4 plasma membrane protein with Nonidet P40. Plasma membranes were purified as described previously with minor modifications¹⁸. The cells were lysed by nitrogen cavitation in Earle's salts buffered at pH 7.2 with 10 mM sodium phosphate (PBES) containing 1 mM PMSF and 10 mM iodoacetamide. Following differential centrifugation, the membrane pellet containing predominantly plasma membrane and endoplasmic reticulum was dispersed in buffer by brief sonication in a bath sonicator (10 × 10 s in a Branson model B-12) and loaded on a sucrose step gradient. The purified plasma membrane fraction was resuspended in PBES at 2–5 mg ml⁻¹ and stored frozen (–20°C) until use. Detergent solubilization was done by resuspending membranes in PBES with vigorous vortexing and adding 1% NP-40 in PBES to give the desired detergent to protein ratio. Protein concentration was held constant at 0.5 mg ml⁻¹. The samples were incubated on ice for 20 min with frequent vortexing and then centrifuged for 45 min at 100,000g. After centrifugation, the supernatants were removed and the insoluble pellets resuspended in PBES. Protein content of the supernatant and pellets was assayed by the method of Lowry *et al.*³³ in the presence of 1% SDS using bovine serum albumin as the standard. Values shown are averages of duplicate determinations. ●—●—, results obtained using two separate P815 membrane preparations. —○—○—, EL4 membrane solubilization.

Treatment of lymphocyte plasma membranes with the non-ionic detergent Nonidet P-40 (NP-40) solubilizes much of the membrane protein, including histocompatibility antigens and surface immunoglobulins, but leaves an insoluble residue which can be pelleted by centrifugation at 100,000g. When solubilization of P815 (a mastocytoma) and EL4 (a thymoma) membranes was examined in more detail, it was found that the amount of protein solubilized increased with increasing detergent up to a detergent to protein ratio of approximately 4:1, which gave 70–80% solubilization (Fig. 1). The remaining 20–30% of the protein remained insoluble at detergent to protein ratios of up to 30:1. The amount of protein remaining was constant above a detergent to protein ratio of 4:1 regardless of the concentration of detergent (from 2–20 mg ml⁻¹). Re-extraction of the insoluble residue with NP-40 resulted in no additional solubilization. Identical results were obtained using Triton X-100 (TX-100) in these experiments and those described below. The detergent-insoluble material formed a tight, translucent pellet on centrifugation at 100,000g. In comparison, the unextracted membranes formed a white, opaque pellet.

Protein composition of the detergent-insoluble fraction

Relative to red cell membranes, the plasma membranes of nucleated cells contain a larger number of proteins. At least 50 membrane polypeptide chains can be resolved by SDS-polyacrylamide gel electrophoresis of P815 plasma membranes (Fig. 2a, lane 1). Examination of the NP-40-soluble and -insoluble fractions of P815 membranes revealed that most of the proteins were completely solubilized while a small number remained selectively insoluble (Fig. 2). The major components of the insoluble fraction were polypeptides having apparent molecular

weights (MWs) of 70,000 (70K), 69,000 (69K), 42,000 (42K), 38,000 (38K), and 36,000 (36K). The p70, p69, p38 and p36 proteins were found only in the detergent-insoluble fraction. Proteins at 42K were present in both the soluble and insoluble fractions. The occurrence of the minor bands seen in the detergent-insoluble fraction, particularly the low-molecular weight proteins near the dye front, varies between membrane preparations. Some of these may be products of proteolysis, although phenylmethylsulphonyl fluoride (PMSF, 1 mM) and iodoacetamide (10 mM) are present during membrane purification and solubilization.

Comparison of membranes and whole cells labelled with ¹²⁵I by lactoperoxidase-catalysed iodination suggested that most of the detergent-insoluble proteins are not exposed in intact cells. p70, p69, p38 and p36 are prominently labelled proteins on isolated membranes (Fig. 2b, lane 4). In contrast, they are not detected when proteins from labelled intact cells, containing comparable amounts of radioactivity, are examined (Fig. 2b, lane 5). p42 is also a major labelled protein on membranes but proteins in the same molecular weight range are labelled on intact cells; therefore, these results do not exclude the possibility that some of the p42 is present at the cell surface. The efficient labelling of p70, p69, p38 and p36 by lactoperoxidase on

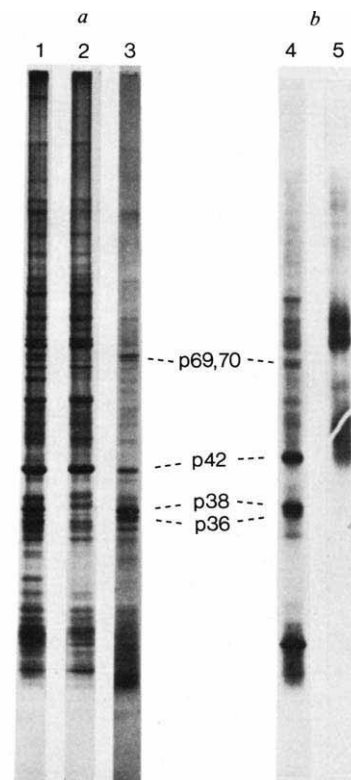
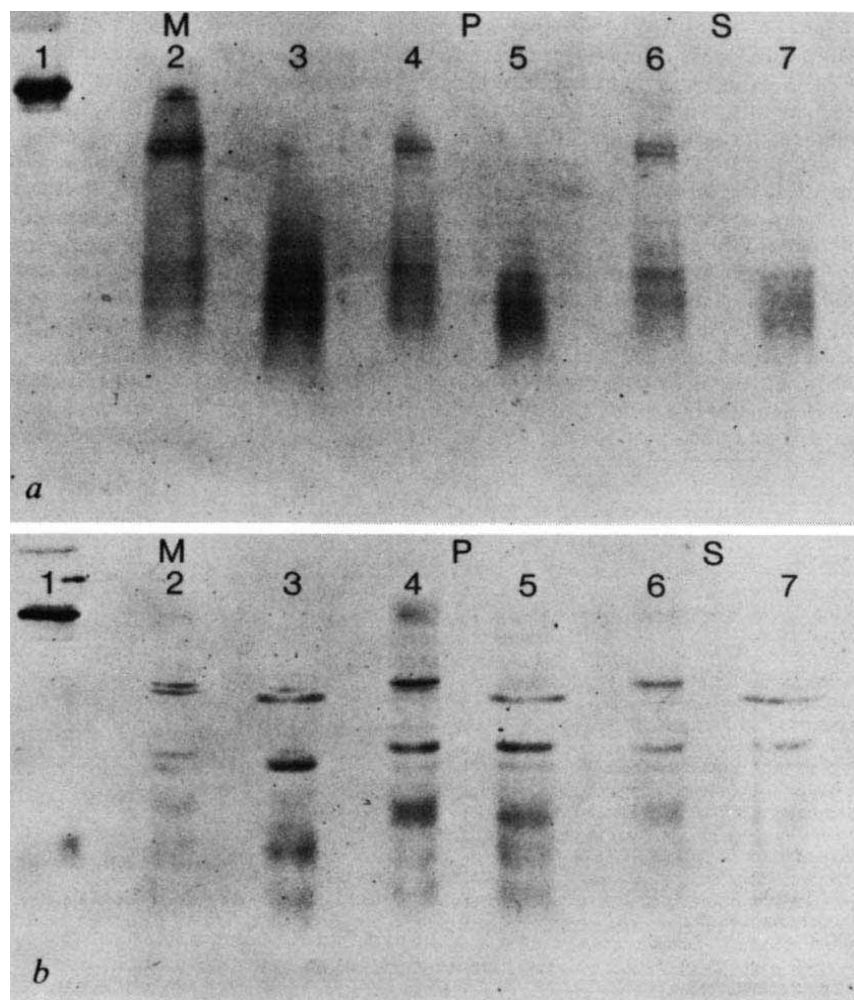


Fig. 2 a, The protein composition of membranes and detergent-soluble and -insoluble fractions. P815 tumour cell membranes were prepared as described in the Fig. 1 legend. Membranes were solubilized in 0.5% NP-40 (also as described in Fig. 1 legend) at a detergent to protein ratio of 5:1 (w/w). The samples were then analysed by SDS-polyacrylamide slab gel electrophoresis following reduction with 5% β-mercaptoethanol. Electrophoresis was done using the buffer system of Laemmli³⁴ on a running gel consisting of a 5–15% polyacrylamide gradient. Gels were stained with Coomassie Brilliant blue following electrophoresis. Lane 1, intact membranes; lane 2, proteins solubilized by 0.5% NP-40 (100,000g supernatant); lane 3, detergent-insoluble fraction (100,000g pellet). b, ¹²⁵I-labelled membranes and intact cells. Cells were labelled by lactoperoxidase-catalysed iodination as previously described³⁵. After labelling and washing to remove free iodine, cells were solubilized in SDS gel application buffer, reduced with 5% β-mercaptoethanol and electrophoresed as described above. Isolated membranes were labelled by the same procedure, washed and prepared for electrophoresis in the same way. Following electrophoresis, the gel was dried and ¹²⁵I-labelled proteins visualized by autoradiography. Lane 4, ¹²⁵I-labelled membranes; lane 5, ¹²⁵I-labelled whole cells. Stained samples (lanes 1–3) and iodinated samples (lanes 4, 5) were run on separate SDS gels. MWs are based on standard proteins run on each gel.

Fig. 3 Limited proteolysis peptide mapping of p42 from the NP-40-soluble and -insoluble fractions of P815 tumour cell membranes. Mapping was done by the procedure of Cleveland *et al.*²². Plasma membranes were solubilized with NP-40, the soluble and insoluble fractions electrophoresed on 7.5% polyacrylamide gels and the gels lightly stained. The p42 bands of each fraction were sliced out and soaked for 30 min in a solution containing 0.125 M Tris-HCl, pH 6.8, 0.1% SDS and 1 mM EDTA. The slices were then placed in the sample wells of a 15% polyacrylamide gel and the same buffer containing 10% glycerol and a given amount of protease layered on top. Electrophoresis was then done in the usual manner with the exception that the current was turned off for 30 min just before the bromophenol blue tracking dye entered the running gel. Gels were stained for protein with Coomassie Brilliant blue. Proteolytic enzymes used were: *a*, *Streptomyces* protease, type VI (Sigma); *b*, V8 protease (Miles) from *Staphylococcus aureus*. Protein samples for mapping were: M, rabbit muscle actin with: no protease (lane 1), 0.05 μ g protease (lane 2) and 0.25 μ g protease (lane 3). P, p42 from the detergent-insoluble fraction (100,000g pellet) with 0.05 μ g (lane 4) and 0.25 μ g (lane 5) protease. S, p42 from the detergent-soluble fraction (100,000g supernatant) with 0.05 (lane 6) and 0.25 μ g (lane 7) protease.



isolated membranes and not on intact cells suggests that these proteins are located on the inner face of the membrane.

Actin has been demonstrated to be present in plasma membranes from a variety of cell types²⁰, including lymphocytes²¹, and the 42K MW protein present in P815 membranes co-migrates with rabbit skeletal muscle actin. When the p42s of the NP-40-soluble and -insoluble fractions were compared by limited proteolysis peptide mapping²², it was found that both gave peptide patterns which confirmed their identity as actin (Fig. 3). Fragments obtained using *Streptomyces* protease (Fig. 3a) were very similar for rabbit muscle actin and p42 and those obtained using V8 protease (Fig. 3b) showed only small differences between the two. Small differences in peptide patterns are expected for the same protein from different species. No significant differences with either protease could be seen for the soluble and insoluble forms of p42. It thus seems that P815 membranes have two forms of actin, one soluble in NP-40 and the other making up a major component of the detergent-insoluble fraction. As mentioned above, re-treating the insoluble fraction with detergent results in no further solubilization of the actin.

Results of limited proteolysis peptide mapping of p38 and p36 ruled out the possibility that one or both of these proteins might be actin degradation products (results not shown). Although p70, p69, p38 and p36 have not been identified, we should point out that they do not have the same molecular weights as any of the proteins which have been shown to interact with actin or to be components of cytoskeletal structures. α -actinin, 100,000 MW, has been suggested to be involved in the attachment of microfilaments to the plasma membrane^{23,24} and Hoessli *et al.*²⁵ have shown that murine lymphocytes synthesize a protein having the expected properties of α -actinin. A protein

of 100,000 MW is present in intact membranes from P815 cells but is completely solubilized by NP-40 (Fig. 2).

A detergent-insoluble component comprising the same major proteins has been found in membranes of all the tumour cell lines examined and in normal murine lymphocytes. Such cells include EL4 (a thymoma), RDM-4 (a lymphoma), CH1 (a B-cell lymphoma) and normal murine spleen lymphocytes. In all cases, NP-40 solubilization at detergent to protein ratios of $>4:1$ leaves 20–30% of the protein insoluble and the major protein components of the insoluble fraction have MWs of 70K, 69K, 42K, 38K and 36K.

The cell-surface enzyme 5'-nucleotidase^{26,27} was also found to be a component of the detergent-insoluble fraction. When P815 membranes were solubilized as shown in Fig. 1, 90–98% of the 5'-nucleotidase activity was recovered in the detergent-insoluble fraction at all detergent to protein ratios tested. This was also true for EL4, CH1 and normal spleen cell membranes. 5'-Nucleotidase purified from pig lymphocytes has been reported to have a MW of 130,000 (ref. 28). It is unlikely, therefore, that any of the major proteins seen in the insoluble fraction is 5'-nucleotidase; the enzyme is probably present in too low an amount to be visualized by Coomassie blue staining.

5'-nucleotidase is present on a wide variety of cell types including most lymphocytes, and cytochemical studies have demonstrated that it is localized predominantly on the cell surface^{26,27}. Experiments were done to confirm the surface location of the 5'-nucleotidase activity of P815 cells. The cells were incubated for 1 h at 4°C in the presence or absence of 5 mg ml⁻¹ concanavalin A (Con A), an inhibitor of 5'-nucleotidase. They were then washed to remove unbound lectin, lysed by freeze-thaw, centrifuged at 100,000g to pellet membranes and the 5'-nucleotidase activity of the pellets measured. Cells

which had been exposed to Con A had about 70% less activity than untreated cells. Control experiments demonstrated that free Con A, which could cause inhibition of internal 5'-nucleotidase, was not available at the time of cell lysis. Following washing to remove unbound Con A, treated and untreated cells were mixed and lysed by freeze-thaw. The 5'-nucleotidase activity of the membranes was the sum of that obtained for each type of cell lysed separately, indicating that no free Con A was available to inhibit the activity of the untreated cell membranes. Similarly, when both types of membrane were mixed and assayed, no further inhibition was obtained. These results indicate that at least 70% of the membrane-bound 5'-nucleotidase activity of P815 cells is located at the cell surface. This probably represents a minimum estimate as cell aggregation during the incubation with Con A may result in some of the surface enzyme becoming inaccessible to the lectin.

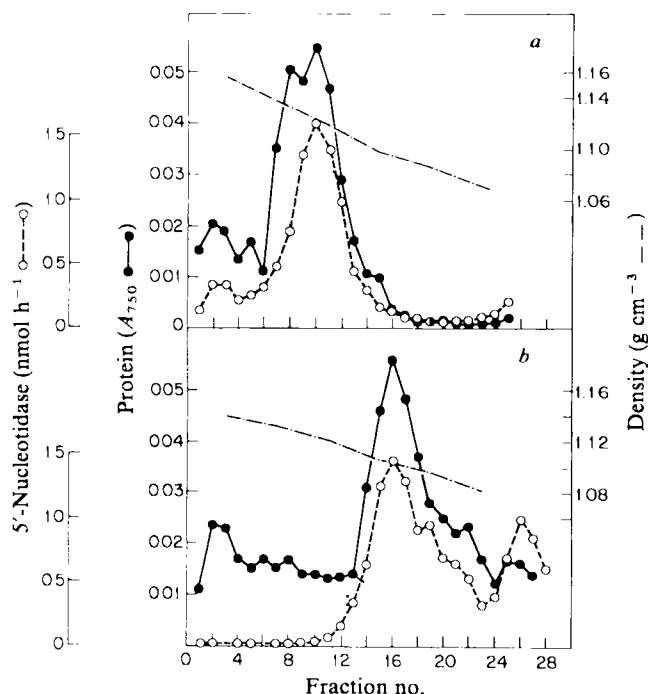


Fig. 4 Sucrose density gradient profiles of P815 plasma membranes and the detergent-insoluble fraction. Detergent-insoluble material was prepared as described in Fig. 1 legend using Triton X-100 in PBES at a detergent to protein ratio of 5:1 and washed once in PBES. Membranes or detergent-insoluble material were resuspended in PBES at a concentration of 2 mg ml⁻¹ and layered on top of a 20–40% sucrose gradient (in 10 mM Tris-HCl, pH 7.2) with a 50% cushion at the bottom. The gradients were centrifuged for 18 h at 18,000 r.p.m. in a Beckman SW-27 rotor. Fractions were then collected from the bottom of the tubes and assayed for protein (A_{750}) and 5'-nucleotidase activity¹⁶. *a*, Density gradient profile obtained with 500 μ g of P815 membranes; *b*, profile obtained with 400 μ g of detergent insoluble material.

Density gradient centrifugation of the detergent-insoluble fraction

The occurrence of a specific set of proteins, including part of the membrane actin, in the NP-40-insoluble fraction suggested that this material might consist of a membrane skeletal network structure and not simply membrane proteins insoluble in NP-40 in aqueous buffer. We therefore examined the insoluble fraction by equilibrium density gradient centrifugation to determine if it behaved as a discrete structure and as an integral part of the membrane and not a contaminant in the membrane preparation.

P815 plasma membranes were found at a density of 1.125 ± 0.001 g cm⁻³ (average of three experiments $\pm$ s.d.) on overnight centrifugation in a 20–40% sucrose gradient (see Fig. 4). When the TX-100-insoluble fraction from P815 membranes was examined in the same way, the protein and 5'-nucleotidase peak

had shifted to a density of 1.107 ± 0.005 g cm⁻³ (average of three experiments $\pm$ s.d.). Most (>70%) of the 5'-nucleotidase activity remained associated with the protein peak. The TX-100-insoluble fraction has a somewhat lower density than intact membranes. Experiments using ³H-labelled TX-100 demonstrated that the lower density was not due to residual detergent bound to the protein. Less than 0.02 mg of detergent per mg of protein could be detected in the washed, detergent-insoluble material. Some of the membrane lipid remains bound to the red cell cytoskeleton on isolation using TX-100⁶ and this is also the case for the detergent-insoluble material from P815 cells. This fraction contains 1.0–1.4 μ mol phospholipid per mg of protein (chloroform/methanol extractable organic phosphate). Preliminary examination of this lipid by TLC indicates that some of the membrane lipids remain selectively associated with the insoluble fraction. Studies are in progress to identify these lipids.

The protein composition of fractions across the TX-100-insoluble peak from a density gradient was examined by SDS-gel electrophoresis (not shown). No indication of any separation of one protein species from another could be detected. Examination of fractions across the intact membrane peak from a density gradient in the same way also gave no indication of any separation of the major insoluble proteins from other membrane components. These results strongly suggest that the proteins of the detergent-insoluble fraction are components of the plasma membrane and form a structure which remains intact after solubilization of the other membrane proteins and much of the lipid. Examination of the insoluble fraction by electron microscopy further supports this suggestion.

The detergent-insoluble plasma membrane matrix

The results described above strongly suggested that plasma membranes have a matrix of protein at their cytoplasmic face which can be isolated on the basis of its insolubility in non-ionic detergent. The detergent-insoluble preparations were examined by electron microscopy to determine if their appearance was consistent with this conclusion. Plasma membranes from P815 cells isolated by the procedure used here are predominantly in the form of closed vesicles¹⁸. They are heterogeneous in size, ranging from ~0.1 to 1.2 μ m in diameter, and have a typical unit membrane appearance. No filamentous material associated with the membranes is apparent in the preparations¹⁸. The detergent-insoluble fraction from these membranes also seems to be largely in the form of closed structures (Fig. 5) also having a heterogeneous size distribution. A unit membrane structure does not seem to be present.

The appearance of the detergent-insoluble fraction in thin section supports the suggestion that this material consists of a skeletal matrix structure isolated from the inner surface of the membrane and not simply of aggregates of insoluble protein. No protein layer adjacent to the bilayer is apparent in thin sections of intact membranes, suggesting that the skeletal network is very closely associated with the lipid bilayer. The spectrin network of red blood cells is similarly not apparent in thin sections of intact red cell membranes⁶.

Further evidence for the presence of a matrix associated with the plasma membrane was provided by experiments done with whole cells. Treatment of intact lymphoid cells with NP-40 (or TX-100) lyses the cells and solubilizes the membrane and cytoplasmic proteins while leaving the nuclei intact. This procedure is frequently used for solubilizing cell-surface antigens before immunoprecipitation. If the detergent-insoluble proteins form a continuous matrix on the inner surface of the plasma membrane, it might be expected that this matrix would be trapped around the nucleus and sedimented by low speed centrifugation along with the intact nuclei following cell solubilization. The 5'-nucleotidase activity associated with the detergent-insoluble matrix provides a convenient marker for determining if this is the case. When P815 cells were solubilized with 0.5% NP-40 (at 5×10^6 cells ml⁻¹) and centrifuged for

10 min at 1,000g, ~60% of the 5'-nucleotidase activity was found in the nuclear pellet. In these same conditions, >95% of 125 I-labelled cell-surface proteins were found in the supernatant. Of the 40% of 5'-nucleotidase present in the low speed supernatant, approximately half (19%) could be sedimented by centrifugation at 100,000g. When the same experiment was done using normal murine spleen cells, essentially identical results were obtained.

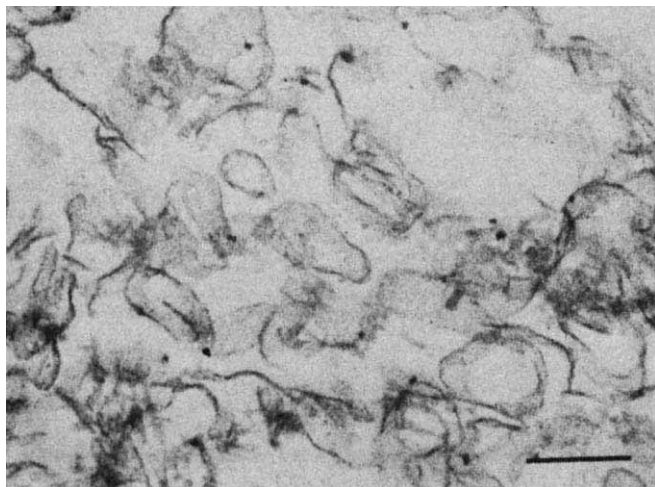


Fig. 5 Electron micrograph of thin section of the detergent-insoluble fraction (100,000g pellet from P815 plasma membrane). Scale bar, 0.2 μ m.

Discussion

The results described here strongly suggest that actin and a small, discrete set of additional proteins form a matrix associated with the plasma membrane of murine tumour and lymphoid cells. Labelling studies using lactoperoxidase indicate that the matrix is probably localized at the inner face of the membrane, although further studies will be necessary to confirm this. The matrix is in some respects similar to the spectrin matrix of red blood cells in that it remains selectively insoluble in non-ionic detergents and some of the membrane lipid remains associated with it. Not surprisingly, the protein components are different from those of the spectrin matrix, with the exception of actin.

Unlike erythrocytes, nucleated cells have extensive internal membrane systems and it is therefore more difficult to obtain pure plasma membranes. Although the plasma membranes used in these experiments are highly purified, it is clear that some contaminants are present, including small amounts of endoplasmic reticulum and some small, electron-dense material¹⁸. Several of the observations reported here argue strongly, however, that the detergent-insoluble matrix is derived from the plasma membrane and not from contaminants in the preparation. The detergent-insoluble matrix proteins account for a relatively large fraction (~20%) of the total protein in the preparations and these proteins co-purify with the plasma membranes on density gradient centrifugation. Although some contaminants are apparent in electron micrographs of the purified membranes, none has the appearance of the isolated matrix. The large size and closed structure of the isolated matrix make it very likely that they are derived from the plasma membrane vesicles. The association of 5'-nucleotidase with the detergent-insoluble matrix also provides strong evidence for the plasma membrane origin of this material. The majority of this enzyme is localized on the plasma membrane of P815 (see results) and other cells^{26,27}.

The functional role of the plasma membrane matrix described here is not known. It may have a structural role, serving to stabilize the membrane bilayer. The location of the matrix also

makes these proteins likely candidates for mediating interactions between transmembrane proteins and the filament systems of the cytoskeleton. The matrix proteins may serve as anchorage points on the inner membrane surface for components of the cytoskeletal system.

In other work being done in this laboratory²⁹ it was found that reconstituted vesicles containing lipid, purified H-2 antigen and the isolated matrix proteins could be formed by mixing the components in deoxycholate-containing buffer and dialysing to remove the detergent. The H-2 antigen present in these vesicles was much more effective in stimulating generation of an *in vitro* cytolytic T-lymphocyte response than vesicles having only H-2 and lipid. Whether this greater effectiveness of the vesicles containing matrix proteins results from interaction of the H-2 with the matrix is now being investigated.

Evidence for the interaction of at least one cell-surface glycoprotein with the matrix is provided by the observation that 5'-nucleotidase remains associated with the detergent-insoluble fraction. This association may occur through interaction of the enzyme with either a matrix protein(s) or with the lipid which remains associated with the matrix. It is difficult to speculate about the functional role for this association, as the role of the cell-surface 5'-nucleotidase activity is not well understood. It is interesting, however, that the cell-surface 5'-nucleotidase displays an unusual property with respect to inhibition by Con A which may be related to its association with the matrix. Carraway *et al.*³⁰ have shown that the 5'-nucleotidase activity of MAT-A and MAT-C1 cells is inhibited in a non-cooperative manner by Con A. Cooperative inhibition was observed, however, if the cells were first treated with colchicine, cytochalasin B or D, or dibucaine. No direct correlation could be found between the induction of cooperativity and changes in Con A receptor redistribution or cell morphology.

The association of 5'-nucleotidase with the matrix and the resulting lack of solubilization when whole cells are lysed with NP-40 suggest a potential problem in attempting to identify cell-surface proteins by immunoprecipitation. Solubilization with non-ionic detergent, centrifugation and immunoprecipitation from the soluble supernatant, has become a standard procedure for determining the cell-surface proteins recognized by antisera and for studying cell-surface antigens. A cell-surface antigen which interacts with the matrix as the 5'-nucleotidase does would be difficult, if not impossible, to isolate by this procedure.

Actin occurs in two forms in the plasma membranes examined here, one soluble in detergent and the other associated with the insoluble matrix. Gruenstein *et al.*³¹ have previously shown that membranes from 3T3 mouse fibroblasts and HeLa cells have two forms of actin. Treatment of the membranes in conditions which favour actin filament depolymerization resulted in removal of 80% (3T3) and 60% (HeLa) of the actin while the rest remained associated with the membrane. This actin fraction may be a component of a membrane matrix similar to that described here. We have noted that the soluble and insoluble forms of actin described here usually have a slight difference in mobility on SDS gels and we are now investigating the possibility that the two forms differ structurally.

With the exception of actin, the protein components of the matrix do not have molecular weights consistent with their being previously described components of the cytoskeletal network. Moore *et al.*³² have examined the TX-100-insoluble fraction from sarcoma 180 ascites cell membranes. The membranes were isolated from cells which had been treated with Zn^{2+} , a procedure which seems to stabilize the interaction of cytoskeletal elements with the membrane. The TX-100-insoluble fraction of these membranes included proteins which the authors tentatively identified as actin-binding protein, α -actinin and actin. Several other polypeptides were also present in these preparations. Our failure to observe components of the cytoskeletal network associated with the membrane matrix may be due to failure to preserve these associations during membrane isolation and/or solubilization. It seems that actin and the other

components observed in our studies are sufficient to form a stable, detergent-insoluble matrix.

Preliminary experiments have suggested that human peripheral lymphocytes have a plasma membrane matrix similar to that found for murine cells. The major protein components had the same mobilities on SDS gels as those found for the matrix components of murine cells. It will be of interest to determine whether a matrix is associated with the plasma

membranes of other cell types (for example fibroblasts) which have more extensive cytoskeletal networks than lymphocytes and, if so, whether the same major protein components constitute the matrix.

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Intrachromosomal gene conversion in yeast

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We have shown that the yeast Saccharomyces cerevisiae has a mechanism by which information from one gene can be transferred non-reciprocally to a repeated copy of the gene on the same chromosome. This intrachromosomal gene conversion may be important in maintaining sequence homogeneity within families of repeated eukaryotic genes.

GENETIC exchange is usually thought to be an inter-chromosomal event occurring between homologous chromosomes in a diploid cell. This exchange of genetic information between two chromosomes may be either a reciprocal or a non-reciprocal (gene-conversion) event. A second type of genetic exchange is intrachromosomal recombination (or conversion). This term has been used to describe both recombination between chromatids attached to the same centromere (sister-strand recombination) and recombination between repeated genes within a single chromatid (intrachromatid recombination). Experimental evidence for both types of intrachromosomal recombination has been obtained. Using a selectable marker inserted into the tandemly repeated ribosomal DNA genes of yeast, T.D.P.¹ and Szostak and Wu² have shown that unequal sister chromatid exchange occurs in meiosis and mitosis. Sister chromatid exchange has also been observed in *Drosophila*³ and in cultured mammalian cells⁴. Reciprocal recombination between repeated elements within a single DNA strand (intrachromatid recombination) has been seen within the 2- μ m plasmid of yeast^{5–9}, as a rare recombination event of yeast chromosome III associated with a mating-type switch¹⁰ and as a mitotic excision of a duplicated *LEU2* gene from chromosome III (ref. 11). No clear example of non-reciprocal intrachromosomal exchange has been reported although mating-type switching in yeast can be interpreted as a unidirectional gene-conversion event^{12,13}.

The technique of yeast transformation provides an opportunity for examining the more general occurrence of intrachromosomal gene exchange. Hinnen *et al.*¹¹ showed that a transforming recombinant plasmid containing an insertion of yeast DNA usually integrates into the yeast chromosome by a homologous recombination event. As shown in Fig. 1 (after Fig. 2 of Hinnen *et al.*¹¹), the result of this integration is a chromosome that contains a non-tandem duplication of a portion of yeast genome with the duplicated segments separated by bacterial plasmid sequences. In the experiments of Hinnen *et al.*¹¹, a recombinant plasmid containing the yeast *LEU2*⁺ gene was integrated into a chromosome containing a *leu2* mutation on chromosome III. We have shown that the mutation in the mutant copy can be transferred to the wild-type *LEU2* gene by an *in vivo* meiotic intrachromosomal gene conversion. We do not know whether the transfer of information occurs as the result of an intrachromatid interaction or an unequal association between sister chromatids. Whatever the details of the interaction, the existence of such a mechanism may have important consequences for the evolution of repeated genes.

Isolation of a strain containing a duplicated *LEU2* gene

The haploid yeast strain AH22¹¹ contains two frameshift mutations, *leu2-3* and *leu2-112*, in the *LEU2* gene on chromosome

III. This strain was transformed with the plasmid CV9 (obtained from J. Hicks). The CV9 plasmid contains a wild-type *LEU2* yeast gene on a *Pst* restriction fragment that is inserted into the *Pst* site of pBR322¹⁴. Forty-nine independent *Leu*⁺ transformants were obtained and one of these, AH22/CV9-12, was analysed in detail.

In the first experiment, AH22/CV9-12 was crossed to a haploid of opposite mating type (strain 2262; ref. 1) that carried a *leu2* mutation. When the resulting diploid was sporulated and tetrad analysis performed¹⁵, 41 of 42 tetrads examined segregated 2⁺:2⁻ for the *Leu2* phenotype. This segregation pattern indicates that the *LEU2*⁺ transforming DNA had integrated at a single site in the host genome¹¹. To determine the site of integration, we crossed AH22/CV9-12 to a *LEU2*⁺ haploid strain HK-3A to form the diploid HK-12. Tetrad analysis of HK-12 showed segregation patterns of 4 *Leu*⁺ to 0 *leu*⁻ spores in more than 90% of the tetrads. This result indicates that the transforming *LEU2*⁺ gene is closely linked to the normal *LEU2* locus on chromosome III. The presumed arrangement of the *LEU2* genes in AH22/CV9-12 is shown in Fig. 1 and the arrangement of markers in the diploid HK-12 (after meiotic DNA synthesis) is given in Fig. 2a. Additional evidence confirming these arrangements will be given below.

Of 306 tetrads analysed from the diploid HK-12, 294 tetrads segregated 4 *Leu*⁺ to 0 *leu*⁻ spores and 12 segregated 3 *LEU*⁺ to 1 *leu*⁻ spores. As described below, a detailed genetic analysis of tetrads showing the aberrant 3⁺:1⁻ pattern provided evidence for intrachromosomal gene conversion.

Genetic analysis of aberrant tetrads

The diploid strain HK-12 had the genotype:

a *leu2-3 leu2-112* plus CV9 insertion

α *LEU2*⁺

<i>his4</i>	<i>TRP1</i> ⁺	<i>ADE6</i> ⁺	<i>MET13</i> ⁺	<i>TYR7</i> ⁺
<i>HIS4</i> ⁺	<i>trp1</i>	<i>ade6</i>	<i>met13</i>	<i>tyr7</i>

For purposes of our analysis, the most important genetic markers other than the *LEU2* genes were the *HIS4* gene, located on chromosome III centromere-distal to *LEU2*, and *TRP1*, a tightly centromere-linked marker on chromosome IV. These markers were used to monitor gene-gene or gene-centromere recombination on chromosome III by standard tetrad analysis¹⁵.

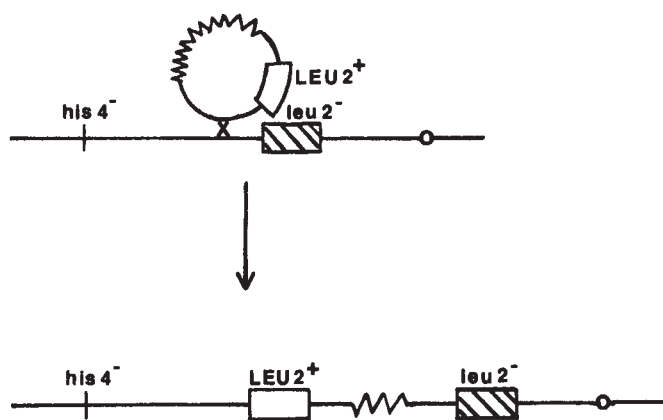


Fig. 1 Derivation of AH22/CV9-12 (after Hinnen *et al.*¹¹). The recombinant plasmid CV9, consisting of a *Pst* fragment carrying the yeast *LEU2*⁺ gene inserted into pBR322, is shown to integrate into the yeast chromosome III during transformation by homologous recombination. The exchange event is shown here to occur in flanking sequences that are centromere-distal to the *LEU2* coding region. The resulting transformant contains duplicated *LEU2* genes separated by pBR322 sequences. The thin line represents yeast sequences, the open boxes the yeast *LEU2*⁺ gene, the lined boxes the yeast *leu2*⁻ gene and the wavy line pBR322 sequences.

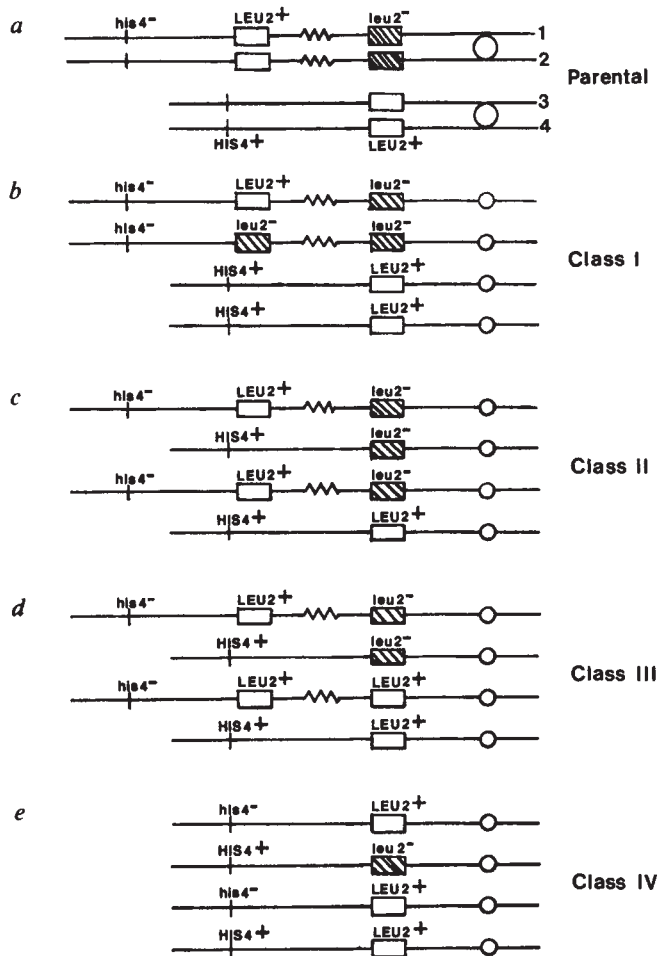


Fig. 2 Arrangement of *LEU2* genes on chromosome III following transformation. The arrangement of *LEU2* genes and the *HIS4* gene on chromosome III in the diploid HK-12 is shown. Also shown are the order of these genes in tetrads from classes I, II, III and IV. a Shows the parental configuration of chromosome III in HK-12 following premeiotic DNA synthesis. The chromatids are labelled 1-4. b Shows an example of the chromosome III configuration in a class I tetrad. In some tetrads, reciprocal recombination events along chromosome III (noted in Table 1) have also occurred. c Shows an example of the chromosome III configuration in the class II tetrads. d Shows the chromosome III configuration in the class III tetrad. e Shows the chromosome III configuration in the class IV tetrad. The thin line represents yeast sequences, the open boxes the *LEU2*⁺ gene, the lined boxes the *leu2*⁻ gene and the wavy line pBR322 sequences.

Each spore clone from the aberrant tetrads was analysed in several ways. First, each strain was checked by standard procedures for auxotrophic requirements. Second, we examined each strain for the presence or absence of pBR322 sequences by using the colony hybridization technique¹¹. Third, DNA from each strain was examined using the Southern blotting technique¹⁶ to determine whether the spore had one or two copies of the *LEU2* gene per chromosome. The details of the Southern analysis are described in Fig. 3 legend.

The procedures described above were done with all the spores from the aberrant tetrads. For some spores, additional tests were done. For those spores that were phenotypically *leu*⁻, we did standard allelism tests¹⁷ to determine whether they contained the *leu2-3* allele, the *leu2-112* allele, both mutant alleles or a new mutation in the *LEU2* gene.

Some of the spores derived from the aberrant tetrads contained two copies of the *LEU2* gene and were phenotypically *Leu*⁺. In such spores, there could be either two *LEU2*⁺ genes or one *LEU2*⁺ gene and one mutant *leu2* gene. To distinguish between these possibilities, we did a mutability test of the spores. The rationale for the test is that it should be more difficult to isolate a *leu2-3* or *leu2-112* mutation from a strain

that has two wild-type *LEU2* genes than from a strain that has one wild-type *LEU2* gene and one *leu2-3 leu2-112* mutant gene. As will be described in more detail below, by comparing the frequency of induced *leu2-3* and *leu2-112* mutations with the frequency of other *leu*⁻ mutations, we could distinguish between two copies of the wild-type *LEU2* gene and one copy each of the wild-type and mutant *LEU2* genes.

Table 1 summarizes the genetic analysis of the aberrant tetrads of HK-12. On the basis of these data, we concluded that there were several mechanisms by which aberrant tetrads could be produced: class I (intrachromosomal gene conversion), class II (interchromosomal gene conversion), class III (reciprocal interchromosomal exchange) and class IV (multiple genetic events).

The four classes of aberrant tetrads

Class I tetrads were the most common aberrant segregation; 6 of the 12 aberrant tetrads were in this group. As described below, our analysis indicates that this class is the result of intrachromosomal gene conversion. The diagnostic feature of class I tetrads is the presence of two mutant copies of the *LEU2* gene

(and associated pBR322 plasmid) in the *leu*⁻ mutant spore (Table 1, Figs 2b, 3). Thus, a chromosome which originally had one mutant *leu2* gene and one wild-type *LEU2* gene has been changed to one which has two mutant genes. Allelism tests showed that the *leu2* mutations present in the *leu*⁻ spores are derived from the *leu2* mutation of the untransformed parent AH22 and do not result from new *leu2*⁻ alleles (Table 1). Both mutant alleles *leu2-3* and *leu2-112* (class IA) or one mutant allele *leu2-3* (class IB) occur in the converted *leu2* gene. The mutational changes during intrachromosomal gene conversion do not involve deletions of any significant size, as no differences in the size of *LEU2*-specific restriction fragments are observed by Southern analysis (Fig. 3). Also, the *leu2*⁻ mutants obtained in class IB can be reverted to the *Leu*⁺ phenotype (H.K., data not shown).

The tetrads of HK-12 that have not undergone a conversion event (Fig. 2a) should have four wild-type *LEU2* genes and two mutant *leu2* genes within the four haploid spores. If class I tetrads represent a gene conversion event of a wild-type *LEU2* gene to a mutant *leu2* gene, they would be expected to have three *LEU*⁺ genes and three mutant *leu2* genes. As previously

Table 1 Genetic analysis and segregation of aberrant tetrads

Class	Tetrad	<i>LEU2</i> *	pBR322	<i>HIS4</i>	<i>TRP1</i>	No. and deduced phenotype of <i>LEU2</i> genes†	Homologous recombination on chromosome III
IA	IA	+	-	+	-	1 (+)	
	B	+	-	+	-	1 (+)	
	C	<i>leu2-3, 2-112</i>	+	-	+	2 (-)	
	D	+	+	-	+	2 (+)	
	2A	+	-	+	-	1 (+)	
	B	+	+	-	+	2 (+)	
	C	<i>leu2-3, 2-112</i>	+	+	+	2 (-)	Exchange between <i>HIS4</i> and pBR322 sequences
	D	+	-	-	-	1 (+)	
	3A	+	-	+	+	1 (+)	
	B	+	+	-	-	2 (+)	
	C	<i>leu2-2, 2-112</i>	+	-	+	2 (-)	Exchange centromere-proximal to pBR322 sequences
	D	+	-	+	-	1 (+)	
IB	4A	<i>leu2-3</i>	+	-	-	2 (-)	
	B	+	-	+	+	1 (+)	
	C	+	-	+	+	1 (+)	
	D	+	+	-	-	2 (+)	
	5A	+	-	+	-	1 (+)	
	B	+	+	-	+	2 (+)	
	C	<i>leu2-3</i>	+	-	+	2 (-)	
	D	+	-	+	-	1 (+)	
	6A	+	+	-	-	2 (+)	
	B	+	-	+	+	1 (+)	
	C	<i>leu2-3</i>	+	-	+	2 (-)	Exchange centromere-proximal to pBR322 sequences
	D	+	-	+	-	1 (+)	
II	7A	+	-	+	+	1 (+)	
	B	+	+	-	-	2 (+)	
	C	+	+	+	-	2 (+)	Exchange between <i>HIS4</i> and pBR322 sequences
	D	<i>leu2-3, 2-112</i>	-	-	+	1 (-)	
	8A	+	+	-	+	2 (+)	
	B	<i>leu2-3, 2-112</i>	-	+	-	1 (-)	
	C	+	-	+	+	1 (+)	Exchange centromere-proximal pBR322 sequences
	D	+	+	-	-	2 (+)	
	9A	+	+	-	+	2 (+)	
	B	+	+	-	-	2 (+)	
	C	<i>leu2-3, 2-112</i>	-	+	-	1 (-)	Exchange centromere-proximal to pBR322 sequences
	D	+	-	+	+	1 (+)	
10A	+	+	+	-	+	2 (+)	
	B	+	-	+	+	1 (+)	
	C	<i>leu2-3, 2-112</i>	-	+	-	1 (-)	Exchange centromere-proximal pBR322 sequences
	D	+	+	-	-	2 (+)	
III	11A	+	+	-	+	2 (++)	
	B	+	-	+	+	1 (+)	
	C	+	+	-	-	2 (+)	Exchange centromere-proximal pBR322 sequences
	D	<i>leu2-3, 2-112</i>	-	+	-	1 (-)	
IV	12A	+	-	-	-	1 (+)	
	B	+	-	+	+	1 (+)	
	C	<i>leu2-112</i>	-	+	-	1 (-)	Exchange centromere-proximal to <i>HIS4</i>
	D	+	-	-	+	1 (+)	

* The *leu2* alleles present in the *leu*⁻ spores were determined by standard allelism tests. These involved mating each *leu*⁻ spore with *leu2-3* and *leu2-112* testers of the appropriate mating type. Following growth of the diploids, the crosses were irradiated with UV rays, grown overnight in the dark and replica-plated to synthetic medium lacking leucine. The appearance of papillations due to mitotic recombination induced by the UV-ray treatment was taken as an indication of non-allelism. When only one *leu2* allele was observed in the *leu*⁻ spore, it is assumed that the second copy of the *LEU2* gene contains the original double mutation *leu2-3 leu2-112*, although it is possible that the *leu2-112* allele may have been lost by intrachromosomal gene conversion.

† This column shows the phenotype of the *LEU2* genes present in each spore. Minus signifies a mutant *leu2* gene. Plus signifies a wild-type *LEU2* gene. This interpretation is based on the data shown in Table 2.

discussed, the only ambiguous spore class in terms of the number of wild-type *LEU2* alleles is that in which the spore is phenotypically *Leu*⁺ and has two copies of the *LEU2* gene. Such spores could contain either two wild-type *LEU2* alleles or one mutant and one wild-type allele. As discussed previously, these two alternatives should be distinguished by testing the frequency with which *leu2-3* and *leu2-112* mutations can be recovered. To do this test, we grew a suspension of the cells being tested in rich medium. The cells were then treated with UV-ray irradiation (75% survival) and grown up overnight in the dark. The culture was then plated for single colonies on rich medium and replica-plated to synthetic medium lacking leucine. Complementation tests and allelism tests were done on the leucine-requiring mutants that were isolated.

The data for spores of class I as well as the original parental strains are shown in Table 2. Two results indicate that all the tested spores from class I tetrads contain one wild-type *LEU2* gene and one mutant *leu2* gene. First, in all cases it was easy to recover mutations at the *LEU2* locus. For each class I strain tested, mutations at the *LEU2* locus accounted for more than half of the total *leu*⁻ mutations. In addition, the spectrum of mutant alleles recovered from the class I spores was similar to those recovered from the parental strain AH22/CV9-12. Second, at least half of the *leu2* mutations produced by UV light are *leu2-3*, *leu2-112* or both *leu2-3* and *leu2-112*. We assume that the UV-ray treatment is not specifically inducing mutations at these loci, but is 'uncovering' the pre-existing mutations. The effect of the radiation is likely to be a stimulation of excision of the wild-type *LEU2* gene or stimulation of gene conversion. The mutant *leu2* genes remaining in the spore after UV-ray treatment are unlikely to be deletion mutants because several of the mutants are revertible (H.K., unpublished data). Also, the *leu2-3 leu2-112* mutants derived from spore HK-121D show recombination with a different *leu2* allele (*leu2-1*).

In summary, the mutability analysis indicates that the class I *Leu*⁺ spores that contain two *LEU2* genes have one wild-type gene and one mutant gene. Within the class I tetrads, therefore, there are a total of three wild-type *LEU2* genes and three mutant *leu2* genes (Fig. 2b). These results strongly support intrachromosomal gene conversion as the mechanism that generates class I tetrads. We do not know whether the intrachromosomal conversion event occurs as the result of an interaction within one chromatid or an unequal interaction between sister chromatids. For either type of interaction, we assume that the conversion event involves heteroduplex formation followed by mismatch repair¹⁸, as for classical gene conversion.

Class II tetrads result from interchromosomal gene-conversion events. They are distinguished from class I tetrads by the segregation pattern of pBR322 sequences with the *leu*⁻ spore. In class I tetrads the *leu*⁻ spore contains pBR322 sequences, whereas the class II *leu*⁻ spore does not (Table 1). Analysis of the types of *leu*⁻ clones generated by UV-ray irradiation of the *Leu*⁺ spores containing pBR322 sequences in the class II tetrads shows that both of these spores in each tetrad contain a mutant *leu2* gene in addition to the wild-type *LEU2* gene (Table 2). Thus, the class II tetrads have three mutant *leu2* genes and three wild-type *LEU2* genes. In contrast to the class I tetrads, the mutant *leu2* genes are distributed over three chromosomes rather than two (Fig. 2c). This pattern of segregation requires the same interchromosomal interaction between homologues as occurs in 'classical' gene conversions¹⁵.

Class III represents a reciprocal recombination event; only one such tetrad was found. In this tetrad, the mutability test of the spore HK12-11A (which is *Leu*⁺ and has two copies of the *LEU2* genes) yields a different result from similar tests of class I and II spores. The low frequency of *leu2* mutations in this strain (Table 2) suggests that the spore HK12-11A has two wild-type *LEU2* genes. Within the four spores, therefore, this class III tetrad has two mutant *leu2* genes and four wild-type *LEU2* genes (as shown in Fig. 2d). The simplest explanation of this segregation pattern is that a reciprocal recombination event occurred between the homologous chromosomes (chromatids 2

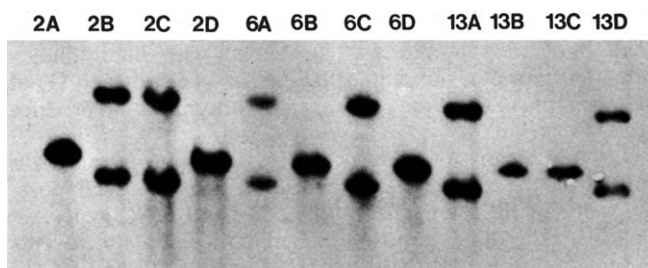


Fig. 3 Quantification of *LEU2* genes. An autoradiogram of two class I tetrads and one control tetrad is shown. Nuclear DNA was isolated²⁹ from class I tetrads 2 and 6 and a control tetrad called tetrad 13 that showed 4⁺:0⁻ segregation for the *LEU2* gene. DNA was digested with *Bam*HI endonuclease. There is no *Bam*HI site within the yeast DNA inserted into plasmid pBR322 in the CV9 vector. The pBR322 plasmid contains one *Bam*HI site. Thus, a yeast strain with one *LEU2* gene should have one *Bam*HI fragment containing the *LEU2* gene. A yeast strain carrying two *LEU2* genes separated by pBR322 sequences will have two *Bam*HI fragments, each containing one *LEU2* gene. Following digestion of nuclear DNA with *Bam*HI, the fragments were separated by agarose gel electrophoresis on a horizontal 0.7% agarose slab gel run at 1 V cm⁻¹ for 36 h. DNA was transferred to nitrocellulose¹⁶. The filters were hybridized to a DNA probe labelled with ³²P by nick translation³⁰. The probe used was the large *Eco*RI fragment of the CV9 recombinant plasmid. The fragment contains approximately one-half of the yeast fragment inserted into pBR322 and half of the pBR322 plasmid.

and 3 in Fig. 2a). This event occurred in the yeast sequences flanked by pBR322 and the mutant *leu2* gene on strand 2 and homologous sequences centromere-distal to the wild-type *LEU2* gene on strand 3 (see also Table 1).

The segregation pattern of this tetrad provides preliminary evidence that the orientation of genes in the AH22/CV9-12 parent is (*his4*)-(*LEU2*⁺ gene)-(pBR322 sequences)-(leu2 mutant gene)-(centromere). If the order of the wild-type *LEU2* gene and mutant gene were reversed, one should not obtain a spore with one mutant *leu2* gene and no pBR322 sequences as the result of a single recombination event.

The most difficult segregation pattern (class IV) to explain is that found for tetrad 12. In this tetrad, no pBR322 sequences are present in any of the spores. In addition, only four copies of the *LEU2* gene are present instead of the expected six copies. As both of the pBR322 sequences are missing from the tetrad, it is possible that two independent meiotic excisions of the plasmid

Table 2 Number of UV ray-induced *leu*⁻ segregants

Strain	<i>leu2-3</i>			<i>leu2</i> mutant alleles different from <i>leu2-3</i> and <i>leu2-112</i>	<i>leu</i> ⁻ complementation groups other than <i>LEU2</i>
	2-112	<i>leu2-3</i>	<i>leu2-112</i>		
1D	18	0	9	5	13
2B	15	10	7	6	21
3B	3	2	0	2	1
4D	18	2	0	3	12
5B	7	0	2	1	4
6A	2	0	6	6	3
7B	11	1	4	3	3
7C	9	3	2	1	2
8A	10	0	0	0	0
8D	0	3	1	10	2
9A	3	0	1	1	2
9B	2	0	0	3	1
10A	1	2	1	2	3
10D	4	1	1	2	4
11A	0	0	0	0	10
11C	16	0	3	2	9
AH22/CV9-12	12	5	7	6	0
HK-3A	0	0	0	0	2

Selected spores from Table 1 that were *Leu*⁺, but contained two copies of the *LEU2* gene were grown overnight in rich medium. Approximately 10⁶ cells were irradiated with UV rays to give 75% survival. Cells were grown overnight in the dark, plated for single colonies on rich medium and replica-plated to synthetic medium lacking leucine. Leucine-requiring mutants were picked and retested for the leucine requirement. The *leu2* alleles were determined by the allelism test used in Table 1.

occurred (one associated with a conversion). Alternatively, if the excision of the plasmid was associated with heteroduplex formation between the duplicated *LEU2* genes during G₁ of meiosis, replication of the resulting chromosome could produce a class IV tetrad.

Our discussion of classes I–IV aberrant tetrads has emphasized the number and types of *LEU2* genes in each spore. By examining the segregation of the *HIS4* and *TRP1* genes, we could also determine the position of other recombination events along chromosome III (between *HIS4* and *LEU2* and *LEU2* and the centromere). This information is also summarized in Table 1.

In conclusion, a non-tandem duplication consisting of a mutant *leu2* gene and a wild-type *LEU2* gene can be altered by a number of genetic mechanisms. The most common alteration is an intrachromosomal gene conversion. Interchromosomal gene-conversion events and other alterations are also found. As will be discussed below, we believe intrachromosomal gene conversion may have important consequences in the evolution of repeated genes.

Discussion

Repeated cycles of unequal sister chromatid exchange have been suggested as a mechanism for maintaining homogeneity among highly repeated sequences such as the ribosomal DNA sequences, satellite DNA, histone and the immunoglobulin genes^{3,19,20}. Recently, this mechanism has been extended to the globin genes following the observation that the restriction enzyme maps of the duplicated α -globin genes in humans and primates are highly conserved within a species^{21,22}. Although unequal sister-strand recombination undoubtedly occurs in some systems^{1,2,22–24}, it is not clear that such exchanges are the mechanism by which sequence homogeneity is maintained. There are at least two inherent disadvantages in maintaining sequence homogeneity among repeated genes by unequal sister-strand exchange. First, unequal sister-strand exchange involves a change in gene dosage. For some classes of repeated genes, therefore, one would expect selection against the products of the exchange. Second, if the repeated genes on a chromosome are not arranged in tandem, but are separated by unique DNA, unequal sister chromatid exchange will result in the deletion of these unique sequences from one chromatid.

We believe that certain experimental results can be most easily explained by an intrachromosomal event. For example, in certain individuals, the γ -globin genes contain a restriction endonuclease pattern which is different from that found in most humans²⁵. Both copies of the γ gene on a single chromosome contain this change. Jeffreys²⁵ has proposed that some type of intrachromosomal recombination event maintains this polymorphism. DNA sequencing of γ -globin genes suggests the possibility of a gene-conversion event²⁶.

Although intrachromosomal gene conversion has the advantage that a mutation can be duplicated or deleted in a single generation without changing gene dosage, it is still possible, of course, that unequal sister-strand recombination is also important in maintaining sequence homogeneity, as these

events can be quite frequent^{1,2}. In the present study six tetrads contained an intrachromosomal gene conversion and there were no examples of an unequal sister-strand exchange. We do not know whether the lack of unequal exchanges is the result of the small sample size, the small size of the duplicated segment or some other factor. We suggest that in some systems, unequal sister-strand exchange may be more important as a mechanism for changing gene dosage than as a mechanism for maintaining sequence homogeneity. The relative importance of intrachromosomal gene conversion and unequal sister-strand exchange in maintaining sequence homogeneity may depend on many factors, for example, whether the duplicated genes are in tandem or separated by unique DNA sequences, the number of repeated units in the family or the DNA sequence within the repeated unit.

In the data reported here, intrachromosomal gene conversion, resulting in two mutant *leu2* genes, was observed in 6/306 unselected tetrads, a frequency of 2%. If the conversion of a mutant *leu2* allele to a wild-type *LEU2* allele is assumed to occur at the same rate, the overall frequency of intrachromosomal gene conversion is estimated to be 4%. This frequency is clearly much higher than the frequency of mutation. Consequently, this mechanism should be sufficient to maintain the homogeneity of certain classes of repeated genes (such as those genes that code for ribosomal RNA) within the yeast genome. Note also that for some classes of repeated genes, intrachromosomal gene conversion may not function often enough to remove all sequence diversity. The conversion frequencies may depend on such factors as the specific base sequence, the size of the repeated gene or the distance between repeated units.

Finally, there is the question of whether the intrachromosomal gene conversion that we have observed occurs naturally in yeast or is induced by the insertion of the recombinant plasmid CV9. Although we cannot entirely rule out the latter possibility, we believe that it is unlikely. Using different recombinant plasmids and different selectable yeast genes, Jackson and Fink (personal communication) and Falco and Botstein (personal communication) have found similar results. In the experiments of Jackson and Fink, both meiotic and mitotic conversion events were observed. In addition, Munz and Leupold²⁷, in reversion studies of suppressor mutations in the yeast *Schizosaccharomyces pombe*, have obtained evidence that is consistent with the occurrence of interchromosomal gene-conversion events among repeated tRNA genes. Scherer and Davis²⁸ have recently demonstrated that similar non-homologous interchromosomal gene-conversion events occur in *Saccharomyces cerevisiae*.

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Ordering of mouse immunoglobulin heavy chain genes by molecular cloning

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We have determined the order of the mouse immunoglobulin $\gamma 1$, $\gamma 2b$, $\gamma 2a$ and ϵ genes by molecular cloning of overlapping chromosomal segments. The results clearly demonstrate that the order is 5'– $\gamma 1$ –(21 kilobases)– $\gamma 2b$ –(15 kilobases)– $\gamma 2a$ –(15 kilobases)– ϵ –3'. There seem to be no J regions at the 5' side of each constant region gene so far obtained except for the μ gene and these constant region genes seem to have repetitive sequences characteristic of switch (S) regions at their 5' side.

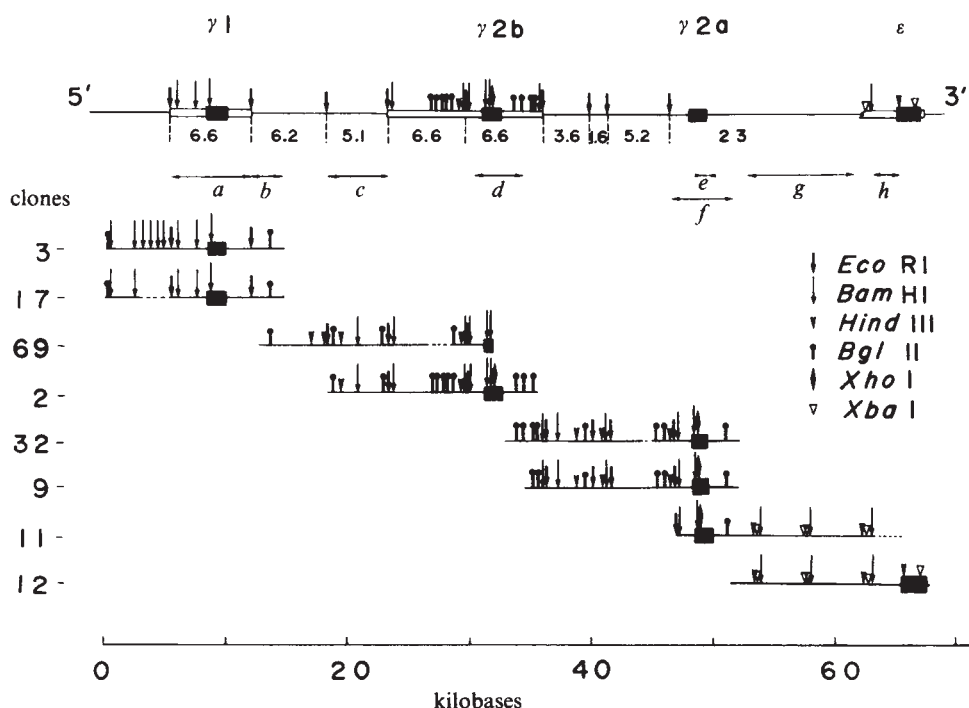
IMMUNOGLOBULIN heavy (H) chain genes comprise a family of variable region (V) genes and several constant region (C) genes which are classified, in mouse, into five major classes: μ , γ , α , δ and ϵ . Genetic studies using allotype markers have clearly demonstrated that the V_H and C_H genes of a single chromosome are coordinately expressed—this is called *cis* expression^{1–3}. During differentiation, a given lymphocyte seems to be able to associate a single V region sequentially with two or more different classes of H chain C region^{4–11}. In this H chain class switch, the direction of the switch is always from μ to γ or α .

Using cDNA–mRNA hybridization kinetics, we have shown that specific C_H genes are deleted in mouse myelomas depending on which C_H genes are expressed¹², and have proposed that a chromosomal segment which intervenes recombining V_H and C_H genes is deleted to bring the V_H and C_H genes close together. Based on the deletion profiles of C_H genes in various myeloma tumours producing different C_H genes, a linear arrangement of C_H genes has been proposed as 5'– μ – $\gamma 3$ – $\gamma 1$ – $\gamma 2b$ – $\gamma 2a$ – α –3'.

Subsequently, more direct evidence has been presented that deletion of the intervening DNA segment accompanies the H chain class switch^{13–18}. Such studies using Southern blot¹⁹ hybridization experiments also support the proposed order of C_H genes.

Comparative studies of rearranged and germ-line H chain genes have led to proposals^{20–26} for a molecular mechanism of H chain class switch. According to this model, a complete H chain gene is formed by at least two types of the recombination event. The first type of recombination takes place between a given V_H , a J_H and a D gene segment, completing a V region gene^{23–25}. The second type of recombination is required to switch a C_H gene and occurs between S_μ and S_γ (or S_α) regions which are located at the 5' flanking regions of respective C_H genes. This model postulates two important structural features in the H chain gene organization: (1) the presence of only one set of J_H region genes in the 5' side to the μ gene and (2) the presence of the S region before each C_H gene.

Fig. 1 Restriction endonuclease cleavage maps of overlapping cloned fragments between $\gamma 1$ and ϵ genes. We used a recombinant phage library⁴² for cloning DNA segments that contain immunoglobulin genes or their flanking regions, except for clone 11. The Charon 4A phage⁴³ library of BALB/c mouse embryo DNA (provided by Dr P. Leder) contained partial *Hae*III digests of embryonic mouse DNA with average length 18 kilobases. These fragments were ligated with *Eco*RI linkers and incorporated into phage DNA. The 20-kilobase insert of clone 11 was partially purified from *Eco*RI digests of RPC5 myeloma DNA by RPC5 column chromatography and agarose gel electrophoresis as described previously²⁷. The partially purified DNA fragments were ligated with the outer fragments of Charon 4A phage DNA. Screening was done on 230 × 230 mm tryptone broth plates containing 5–20 × 10⁴ plaques by an *in situ* hybridization method⁴⁴. Appropriate restriction endonuclease fragments of immunoglobulin genes were labelled with ³²P by nick translation⁴⁵ to a specific activity of 30–200 c.p.m. per pg and used as hybridization probes. Nitrocellulose filters were washed as described elsewhere¹⁷. A plaque containing an immunoglobulin gene or its flanking region was picked up and the phages were grown in liquid cultures to prepare DNA as described⁴⁶. At the top is a schematic representation of the chromosomal segment containing the $\gamma 1$, $\gamma 2b$, $\gamma 2a$ and ϵ genes. Open bars indicate DNA segments cloned previously: the $\gamma 1$ gene (IgH22)²⁷, the $\gamma 2b$ gene (IgH22, Ig $\gamma 2b$ –26)^{28,30,33} and the ϵ gene (Ige–1)³⁴. Structural genes are shown as solid boxes with direction of transcription from left to right, determined by nucleotide sequence determination. Numbers under the top line indicate sizes of *Eco*RI fragments in the germ-line gene. Horizontal arrows indicate fragments used as probes for screening or Southern hybridization. The insert of each clone is shown by a horizontal line with restriction sites. Clones were aligned by restriction endonuclease cleavage mapping and Southern hybridization analysis (see text and Figs 2, 4). Restriction digests were examined by electrophoresis in 0.5–0.7% agarose gels. It is possible that very small fragments (for example, 100 base pairs) may have been missed. *Bgl*II and *Xho*I sites at the 3' side of the $\gamma 2a$ gene and *Xba*I sites at the 5' side of the $\gamma 2a$ gene were not determined. Broken lines in clones 11, 17, 32 and 69 indicate deletions.



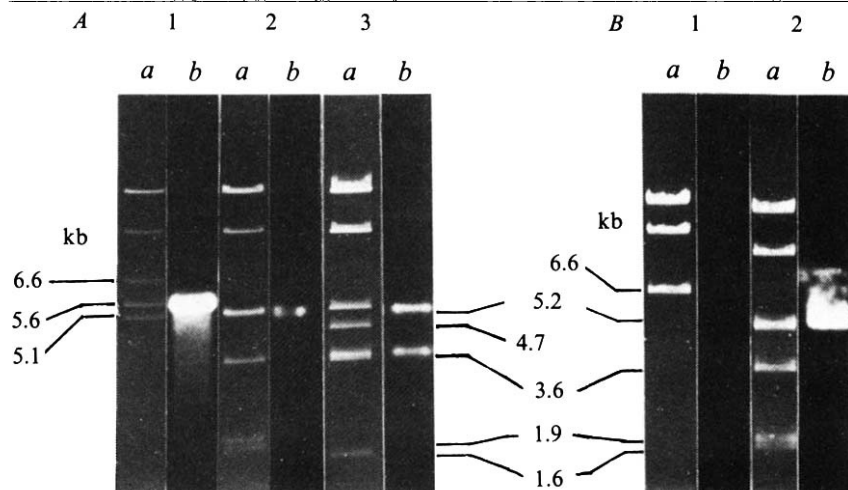


Fig. 2 Southern hybridization of isolated clones linking between the $\gamma 2b$ and $\gamma 2a$ genes. DNAs from isolated clones (0.1–0.3 μ g each) were digested with *Eco*RI and electrophoresed in 0.5% agarose gels. The fragments were transferred to nitrocellulose filters¹⁹ and hybridized with ³²P-labelled probes as described elsewhere¹⁷. Ethidium bromide stains (a) and autoradiograms (b) of Southern blots are shown. In A, 4.0-kilobase (kb) *Xba*I–*Hha*I fragment of $\gamma 2b$ gene (Fig. 1d) was used as a probe. Lanes 1, 2 and 3 contain DNAs of clones 2, 9 and 32, respectively. In B, $\gamma 2a$ cDNA clone (pG2a–14) (probe e in Fig. 1) was used as a probe. Lanes 1 and 2 contain DNAs of the 6.6-kilobase $\gamma 2b$ gene clone (IgH22)^{28,30} and clone 9, respectively.

To determine directly the order of C_H genes and test the proposed C_H gene organization, we and others cloned mouse immunoglobulin genes, and have determined the complete nucleotide sequences of the $\gamma 1$, $\gamma 2b$ and μ genes^{27–31}. Liu *et al.*³² isolated a clone which contains both μ and δ genes, and demonstrated that the μ gene is located ~4.5 kilobases 5' to the δ gene. The linkage of 5'– $\gamma 1$ – $\gamma 2b$ –3' was shown by cloning overlapping chromosomal segments²⁰. We have recently cloned the $\gamma 3$ and ϵ genes^{33,34}, and using these cloned DNA segments as probe, cloned overlapping chromosomal segments from embryonic mouse DNA. Characterization of these clones unequivocally demonstrates that the mouse immunoglobulin γ chain genes are aligned as proposed¹² (that is, 5'– $\gamma 1$ – $\gamma 2b$ – $\gamma 2a$ – ϵ –3') on a chromosome. The linkage of 5'– ϵ –3' is described elsewhere³⁴.

The $\gamma 2b$ gene is located 5' to the $\gamma 2a$ gene

When we screened a Charon 4A library containing partial *Hae*III digests of embryonic mouse DNA with the 4-kilobase *Xba*I–*Hha*I fragment (probe d in Fig. 1) of the $\gamma 2b$ gene clone^{28,30} as a probe, we isolated three clones which were designated Ch · M · Ig $\gamma 2b$ –2 (clone 2), Ch · M · Ig $\gamma 2a$ –9 (clone 9) and Ch · M · Ig $\gamma 2a$ –32 (clone 32). These were characterized by restriction enzyme cleavage mapping and Southern blot

hybridization (Figs 1, 2). Clone 2 contains an *Eco*RI fragment (5.6 kilobases) that hybridizes with probe d (Fig. 2A, lane 1) and shares common restriction sites with the 6.6-kilobase *Eco*RI fragment of the $\gamma 2b$ gene, the nucleotide sequence of which has already been determined³⁰. Comparison of restriction maps of clone 2 and the $\gamma 2b$ clone indicates that clone 2 contains the $\gamma 2b$ gene at the 3' end (Fig. 1).

However, restriction cleavage site mapping indicates that clone 9 does not contain any region corresponding to the $\gamma 2b$ structural gene (Fig. 1), but has one *Eco*RI fragment (5.2 kilobase) that hybridizes with the $\gamma 2b$ gene fragment (probe d) as shown in Fig. 2A, lane 2. Because the $\gamma 2b$ and $\gamma 2a$ genes are known to share extensive homology and cross-hybridize^{30,35,36}, we thought that clones 9 and 32 could contain the $\gamma 2a$ gene.

To test this we used as probe a $\gamma 2a$ cDNA clone pG2a–14 (probe e in Fig. 1) which does not hybridize with the $\gamma 2b$ gene in the stringent conditions used¹⁷. As shown in Fig. 2B, clone 9 was shown to contain an *Eco*RI fragment (5.2 kilobases) that hybridized to the $\gamma 2a$ gene probe (probe e). This *Eco*RI fragment is located at the 3' end of the clone 9 insert and is cleaved by *Xho*I (data not shown). We have determined a partial nucleotide sequence of the 5.2-kilobase *Eco*RI fragment of clone 9. The nucleotide sequence from the *Bam*HI site towards the *Xho*I site of the $\gamma 2a$ coding region is

GGATCCCTGTCCAGTGGTGTG
CACACCTTCCCAGCTGTCTGCAG,

which coincides with that reported for the CH1 domain of the $\gamma 2a$ cDNA clone³⁶. The amino acid sequence

Gly-Ser-Leu-Ser-Ser-Gly-Val-His-
Thr-Phe-Pro-Ala-Val-Leu-Gln

predicted from the nucleotide sequence agrees well with that previously determined for the CH1 domain of the $\gamma 2a$ chain of MOPC173 (ref. 37). The sequence determines the direction of transcription of the $\gamma 2a$ gene and unequivocally demonstrates that clone 9 contains the $\gamma 2a$ gene at the 3' side.

The restriction map analysis indicates that clones 9 and 32 are almost identical to each other except that clone 32 extends 1.7 kilobases further in the 5' direction and has a small deletion (0.5 kilobases) in the middle (Fig. 1). It is clear that clone 32 has a $\gamma 2a$ gene fragment at the 3' end identical to that of clone 9. In addition, clone 32 contains, at the 5' end, a 3.6-kilobase *Eco*RI fragment which hybridizes with the $\gamma 2b$ gene probe (Fig. 2A, lane 3). The restriction map of the 5'-terminal portion (~2.6 kilobases) of clone 32 is indistinguishable from that of the 3' end of clone 2 (Fig. 1). These results indicate that clone 32 shares overlapped regions at the 5' end with clone 2 and at the 3' end with clone 9. The linear alignment of clones 2, 32 and 9 clearly demonstrates that the $\gamma 2b$ gene is located 5' to the $\gamma 2a$ gene and the two genes are 15 kilobases apart (Fig. 1).

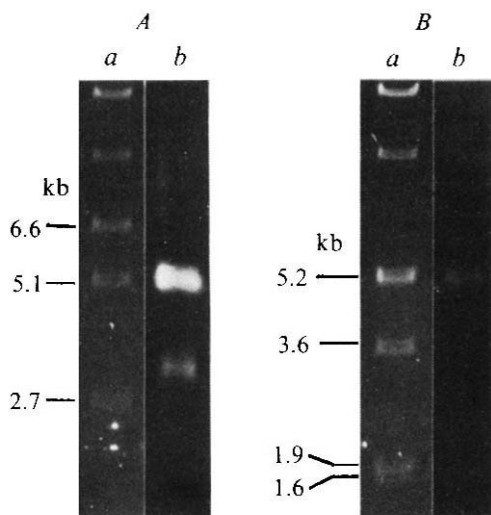
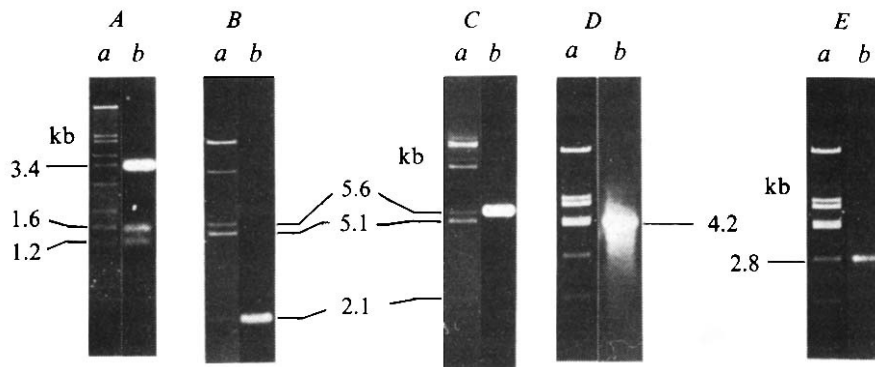


Fig. 3 Cross-hybridization between $S_{\gamma 1}$ and $S_{\gamma 2a}$ regions. Experiments were done as described in Fig. 2 legend. DNA was digested with *Eco*RI and Southern blot hybridized with ³²P-labelled 5' *Eco*RI fragment (5.1 kilobases) of clone 3. Ethidium bromide stains (a) and autoradiograms (b) of Southern blots are shown. A, clone 3 DNA. B, clone 9 DNA. A faint band of 3.1 kilobases in lane A(b) is due to deletion of the 5.1-kilobase fragment of clone 3 during propagation.

Fig. 4 Southern hybridization of isolated clones linking between the $\gamma 1$ and $\gamma 2b$ genes and between the $\gamma 2a$ and ϵ genes. Experiments were done as described in Fig. 2 legend. Ethidium bromide stains (a) and autoradiographs (b) of Southern blots are shown. In A, clone 3 DNA was digested with *EcoRI* and *BamHI*, and the 6.6-kilobase *EcoRI* fragment of the $\gamma 1$ gene (Fig. 1a) was used as a probe. In B, clone 69 DNA was digested with *EcoRI* and 4.0-kilobase *XbaI-HhaI* fragment of the $\gamma 2b$ gene (Fig. 1d) was used as a probe. In C, clone 69 DNA was digested with *EcoRI* and 2.7-kilobase 3' *EcoRI* fragment of clone 3 (Fig. 1b) was used as a probe. In D, clone 12 DNA was digested with *EcoRI* and *BamHI*, and the probe was 2.2-kilobase *BamHI-HindIII* fragment of the ϵ gene (Fig. 1h). E, clone 12 DNA was digested with *EcoRI* and *BamHI* and the probe was the 5.2-kilobase *EcoRI* fragment of clone 9 (Fig. 1f).



The $\gamma 1$ gene is located 5' to the $\gamma 2b$ gene

The embryonic mouse DNA library was screened with a 6.6-kilobase $\gamma 1$ gene fragment (IgH2) cloned previously²⁷ as a probe (probe a in Fig. 1) and two different clones were obtained: Ch · M · Ig $\gamma 1$ -3 (clone 3) and Ch · M · Ig $\gamma 1$ -17 (clone 17). Restriction maps of these are shown in Fig. 1.

Comparison of the restriction maps of clones 3 and 17, and the $\gamma 1$ gene clone (IgH2) indicates that both clones 3 and 17 contain the 6.6-kilobase *EcoRI* fragment of the $\gamma 1$ gene. The comparison also allows determination of the orientation of the two clones. The insert of clone 17 is ~2 kilobases shorter than that of clone 3 and the deletion is located in the 5'-terminal *EcoRI* fragment. These two clones may be derived from a common ancestral phage and the deletion may have occurred during propagation of phages, because the directions of the inserts within these phages and the ends of the inserts in these clones are identical to each other. Furthermore, clone 3 underwent deletion during propagation, producing a phage similar to clone 17 (see Fig. 3A). Southern blot experiments (Fig. 4A) provide additional evidence that clone 3 contains the $\gamma 1$ gene. When clone 3 DNA was digested with *EcoRI* and *BamHI* simultaneously, clone 3 DNA produced 3.4-, 1.6- and 1.2-kilobase fragments which hybridize to the $\gamma 1$ gene (probe a), as expected from the restriction map.

Because $\gamma 1$ gene clones (3 and 17) and $\gamma 2b$ gene clone (2) do not share a common region, we screened the embryonic mouse DNA library with a 3' fragment of clone 3 (Fig. 1, probe b) and a 5' fragment of clone 2 (Fig. 1, probe c) as probes. We isolated a new clone designated Ch · M · Ig $\gamma 2b$ -69 (clone 69)—the restriction map of this is shown in Fig. 1.

The 3' part of clone 69 is indistinguishable from that of clone 2 except that the former has an ~1.5-kilobase deletion at the 5' side of the $\gamma 2b$ gene. Comparison of restriction maps of clones 69 and 2 indicates that clone 69 contains the $\gamma 2b$ gene at the 3' end. Southern blot hybridization experiments show that a 2.1-kilobase *EcoRI* fragment of clone 69 hybridizes with the $\gamma 2b$ gene probe (Fig. 4B). Clone 69 also contains a 5.6-kilobase *EcoRI* fragment that hybridizes with the 3'-terminal fragment of clone 3 (Fig. 1, probe b) as shown in Fig. 4C. From these results it is clear that the 3' region (2.1 kilobases) of clone 3 and the 5' region (2.1 kilobases) of clone 69 are identical. The linear alignment of clones 3, 17, 69 and 2 clearly demonstrates that the $\gamma 1$ gene is located 5' to the $\gamma 2b$ gene and the two genes are 21 kilobases apart. These results are consistent with those of Maki *et al.*²⁰.

The ϵ gene is located 3' to the $\gamma 2a$ gene

We recently cloned a $\gamma 2a$ gene fragment from *EcoRI* digests of RPC5 myeloma DNA designated Ch · M · Ig $\gamma 2a$ -11 (clone 11). Southern blot hybridization and R-loop mapping show that the $\gamma 2a$ gene is located at the 5' end of the 20-kilobase cloned fragment (data not shown). The restriction map of clone 11 is shown in Fig. 1. The 5' region of the RPC5 $\gamma 2a$ gene fragment shares common restriction sites with the 3' region of clones 32 and 9.

The 8.5-kilobase *HindIII* fragment (probe g) of clone 11 was recloned into pBR322 and used as a probe to screen the mouse embryonic DNA library. We obtained a germ-line $\gamma 2a$ gene fragment designated Ch · M · Ig ϵ -12 (clone 12)—the restriction map of this is also shown in Fig. 1. The 16.5-kilobase insert of clone 12 has restriction sites almost identical to those of the 3' portion of clone 11 except that the latter has a deletion at the 3' part.

Cloning of an expressed ϵ gene from an ϵ chain-producing hybridoma has been reported elsewhere³⁴. Partial nucleotide sequence determination indicates that our ϵ gene clone Ch · M · Ig ϵ -1 encodes an amino acid sequence similar to that of human ϵ chain. The expressed ϵ gene (Ch · M · Ig ϵ -1) contains, as do other expressed H chain genes²⁰⁻²⁶, at least three germ-line gene segments: a V gene, a J gene (including S_{μ} region that flanks the 5' end of the μ gene) and the ϵ gene. The restriction map of the region encoding the ϵ gene of Ch · M · Ig ϵ -1, shown at the top of Fig. 1, seems to be identical to that of the 3' region of clone 12.

To test whether or not clone 12 contains the ϵ gene, Southern blot hybridization experiments were done using a *BamHI-HindIII* fragment (probe h) of the ϵ gene as a probe. Figure 4D shows that probe ϵ hybridizes a 4.2-kilobase *BamHI + EcoRI* fragment at the 3' part of clone 12. We also confirmed (Fig. 4E) that the 5' segment of clone 12 has a region identical to the 3' segment (probe f) of clone 9 which contains the germ-line $\gamma 2a$ gene. Taken together, these results indicate that the ϵ gene is located 3' to the $\gamma 2a$ gene and the two genes are about 15 kilobases apart.

We have recently isolated three clones³⁸ which link the $\gamma 1$ gene (clone 3) and the $\gamma 3$ gene (Ig $\gamma 3$ -30)³³. Our data indicate that the $\gamma 3$ gene is located 34 kilobases 5' to the $\gamma 1$ gene, and we

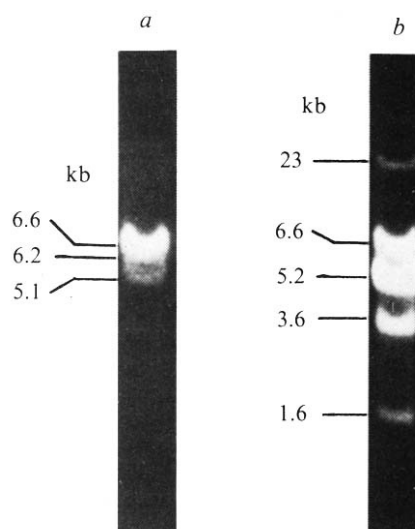


Fig. 5 Distance between the $\gamma 1$, $\gamma 2b$ and $\gamma 2a$ genes. BALB/c newborn mouse DNA (3 μ g) was digested with *EcoRI* to completion and run on 0.5% agarose gel electrophoresis. DNA was transferred to a nitrocellulose filter¹⁹ and hybridized with ³²P-labelled probes as described elsewhere¹⁷. Total DNAs of clones 69 and 9, respectively, were used as probes in a and b.

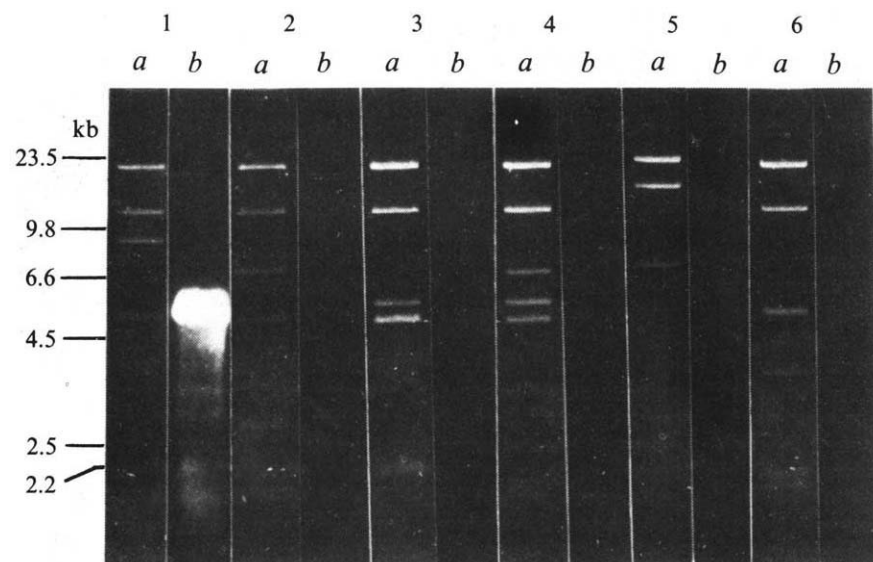


Fig. 6 Hybridization analysis with J-region probe. DNAs from isolated clones covering the region between the $\gamma 1$ and $\gamma 2a$ genes were digested with *EcoRI* and run on 0.7% agarose gel electrophoresis. DNAs were transferred to a nitrocellulose filter¹⁹ and hybridized with the 1.5-kilobase *HindIII*-*EcoRI* fragment that is located 6.5 kilobases 5' to the μ gene. This fragment contains the J_{H4} region²⁴. Ethidium bromide stains (a) and autoradiographs (b) of Southern blots are shown. DNA in each lane is as follows: (1) the expressed $\gamma 1$ gene of MC101 myeloma (Ig $\gamma 1$ -706)^{25,47,48} that contains V, D, J₃, J₄, S _{μ} and C $\gamma 1$ gene segments; (2) clone 3; (3) clone 69; (4) clone 2; (5) the 6.6-kilobase $\gamma 2b$ gene clone (IgH22)^{28,30}; (6) clone 9.

have demonstrated³⁴ that the ϵ gene is present 12.5 kilobases upstream of the α gene. The linkage of J_H - μ - δ has been shown elsewhere^{24,32}. Thus it is now clear that the organization of the mouse C_H gene is 5'- J_H -(6.5 kilobases)- μ -(4.5 kilobases)- δ -(unknown distance)- $\gamma 3$ -(34 kilobases)- $\gamma 1$ -(21 kilobases)- $\gamma 2b$ -(15 kilobases)- $\gamma 2a$ -(14.5 kilobases)- ϵ -(12.5 kilobases)- α -3'. Note that all the C_H genes ordered have the same orientation and thus are transcribed from the same strand of DNA.

Distance between C_H genes in germ-line DNA

Comparison of the restriction maps of the clones isolated indicate that some have deletions which probably occurred during propagation of phages. This was also reported for globin gene clones³⁹. We therefore compared the sizes of *EcoRI* fragments obtained from cloned γ genes and from Southern blot hybridization of newborn mouse DNA. When the total insert of clone 69 was used as a probe, *EcoRI* digests of newborn mouse DNA produced 6.6(doublet)-, 6.2- and 5.1-kilobase bands which coincide with the *EcoRI* fragments deduced from the cloned DNAs (Fig. 5). When the total insert of clone 9 was used as a probe, *EcoRI* digests of newborn mouse DNA produced 23-, 6.6-, 5.2-, 3.6- and 1.6-kilobase bands which coincide with the *EcoRI* fragments deduced from the cloned DNAs.

Furthermore, consideration of the maximum size of the insert (21 kilobases) that can be taken by Charon 4A phage makes it unlikely that there is a deletion >2 kilobases in the clones obtained. Because the insert sizes of clones 69 and 32 are 19.3 and 19.2 kilobases, respectively, the original inserts cannot be more than 2 kilobases greater than those of the present clones. We therefore conclude that the distances between the $\gamma 1$ and $\gamma 2b$ genes and between the $\gamma 2b$ and $\gamma 2a$ genes in the germ-line chromosome are 21.2 and 15.5 kilobases, respectively. Similar experiments using the ϵ gene as a probe³⁴ indicate that the distance between the $\gamma 2a$ and ϵ gene is 14.5 kilobases in newborn mouse DNA.

Absence of J region sequences in the entire region encompassing γ and ϵ genes

We determined whether or not each γ gene clone contains a sequence homologous to a J region. We used a J segment (J_4) as a probe and hybridized it with restricted DNA fragments of all the γ gene clones shown in Fig. 1. There were no J or J-like sequences between 8 kilobases 5' to the $\gamma 1$ gene and 3 kilobases 3' to the $\gamma 2a$ gene (Fig. 6). The J_4 sequence was actually expressed in the $\gamma 2b$ chain produced by MOPC141 myeloma²⁴. It can be argued that other completely different J sequences may be present in the γ gene-flanking regions. If this is the case, V region sequences formed by the use of J_μ and J_γ are different. This is unlikely because the same idio type (a V region sequence) can be expressed as either μ or γ chain. It is possible that a γ

gene has completely different J-flanking regions and an identical J_4 sequence of 51 base pairs which was not detected by our probe. This also seems unlikely because the precise joining between the same V gene and J_γ probably requires a nucleotide sequence similar to that of the J_μ -flanking region^{23,24}. A similar experiment was done on the ϵ gene clone (clone 12) and a $\gamma 3$ gene clone³³, in which we did not find a J region or J-like sequences (data not shown).

The S region before each C_H gene may comprise repetitive sequences

The class-switch recombination takes place at the region 5' to each C_H gene. Such regions responsible for the class switch have been functionally defined as S regions²¹. We have recently demonstrated that the S $\gamma 1$, S $\gamma 2b$ and S $\gamma 3$ regions comprise tandem repetitive conserved sequences of 49 base pairs^{25,33}. The 5'-flanking region of the $\gamma 2b$ gene which contains the repetitive sequence is deleted from clone 69. Such tandem repetitive sequences of conserved 49-base pair units can be easily deleted when propagated in bacteria⁴⁰. Deletions of short segments (0.5–2.0 kilobases) found in several clones are always located in the 5'-flanking region of each γ gene. The distance from the 5' end of each γ gene to a deleted region is almost constant (~3 kilobases). During propagation of clone 9, we also found a deletion of ~1.5 kilobases at ~3 kilobases 5' of the $\gamma 2a$ gene, about the same place where deletion occurred in clone 32. The deletions in these clones may indicate the positions of repetitive sequences.

Although we have not determined the nucleotide sequence of the S $\gamma 2a$ region, the deletion in clone 32 may suggest a similar repetitive sequence in the 5'-flanking region of the $\gamma 2a$ gene. We found that the 5'-flanking region of the $\gamma 2a$ gene cross-hybridizes with the S $\gamma 1$ region (Fig. 3). The S μ and S α regions also contain repetitive sequences^{22,31,41} and these are often deleted during cloning. Thus it is likely that each C_H gene, except for the δ gene which is probably expressed by RNA splicing³², has its own S region for class-switch recombination and that all the S regions comprise repetitive sequences.

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LETTERS

Variability of the double quasar 0957 + 561 A, B

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Evidence suggests that the double nature of the quasar 0957 + 561 A, B results from the influence of an intervening gravitational lens on the light from a more distant, single quasar. The nearly identical spectral characteristics of the two objects have been established¹⁻³ and have been the incentive for interpreting the two apparent objects as images of a single one. We report here spectroscopic and photometric observations which show that the images of the double quasar 0957 + 561 A, B are variable. Brightness changes were observed to be accompanied by corresponding spectral variations. These data suggest that the quasar itself is an intrinsically variable object.

Photographs by one of us (W.C.K.) taken with the 0.9-m Lick Observatory Crossley reflector on 18 and 19 May 1980 (all dates are given in UT) showed that ratio of brightness in the blue of B to A was 0.95, considerably different from the value of about 0.75 given earlier^{1,4} (A is taken as northern image) and it seemed more likely that B brightened than that A faded⁵. To investigate further the nature of the variability, we obtained additional Crossley photographs and made spectrophotometric observations with the Lick Observatory 3-m Cassegrain image-tube scanner on 9 June 1980. We also obtained spectrophotometric data for the pair on 12, 13 and 14 March 1980 using a similar observing set-up. The instrument and observing procedures have been described elsewhere⁶.

Plates were obtained with the Crossley reflector on 19 May and 7 and 21 June 1980. The plate-filter combination, 103a-0 plus GG 13, corresponds approximately to the B bandpass. We also have a plate taken on 20 March with the Lick Observatory 20-inch astrophotograph for the proper motion program which recorded the double quasar by chance; this plate had roughly the same spectral response as the Crossley plates. Lacking the extension of a photoelectrically calibrated sequence of stars to magnitudes comparable with those of the quasar images, we present here only flux ratios. The ratios, given in Table 1, were determined from IRIS photometry of the plates. We extrapolated the slope of the IRIS photometry calibration to the brightness of the double quasar, which was ~2 mag fainter than our faintest calibrated star. Only a small additional error is

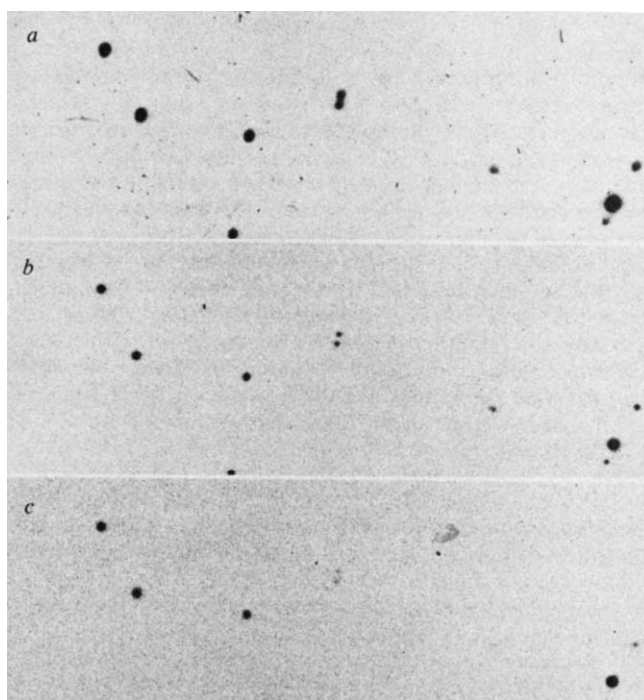


Fig. 1 Three photographs of the double quasar taken with the Lick Observatory Crossley reflector. *a*, 22 January 1915: the images are slightly comatic because the double was not centred on the original plate. *b*, 18 May 1980. *c*, 21 June 1980: this plate was underexposed because of poorer observing conditions, but shows B brighter than A. In all pictures north is at the top and east is at the left, so that image A is the upper of the double; the double is close to the centre of each of the three.

introduced by this procedure when measuring ratios close to unity, and this is included in the errors quoted in Table 1. These ratios refer almost entirely to the continuum fluxes, because the only strong line included, C III] λ 1,909, has a small equivalent width compared with the total bandpass of the plate-filter combination. Also, these measures are only very slightly affected by the presence of the lens galaxy near component B. All measures used comparable IRIS openings corresponding to ~3 arc s diameter, thus including much of the galaxy light with the southern quasar image. This introduced a constant additional contribution, which, from the red magnitude given by Young *et al.*⁴ and an approximate *K*-correction, we estimate to be ~3% of the image B flux; this small correction is not

Table 1 0957+561 A, B brightness ratios (B/A)

Date (UT)	Ratio
20 March 1980	$0.84 \pm .07$
19 May 1980	$0.95 \pm .05$
7 June 1980	$1.15 \pm .07$
21 June 1980	$1.09 \pm .07$

important for the results in Table 1 and has not been included. All exposures were well below the level at which saturation and growth of the galaxy image could affect the measured magnitudes. Figure 1 reproduces two of the 1980 Crossley plates. Observing conditions were less satisfactory on 7 June, resulting in a plate which reproduced poorly, but the images contain sufficient information to provide a reliable flux ratio.

Lick Observatory files contain Crossley plates taken in 1902, 1903 and 1915 which recorded the double quasar, with the plates centred on the galaxy NGC3079. Figure 1 reproduces the plate taken on 22 January 1915 which shows the brightness ratio of the double near that given previously^{1,3,4}. The other archival Crossley plates exhibit a similar flux ratio for the pair, but preliminary analysis indicates that both images were systematically brighter in the past. A detailed analysis of the past history of the images on the archival plates is in progress and will be presented elsewhere.

Additional information on the variability is available in the spectrophotometry. Because we used a 2.8 arc s circular entrance aperture for both the March and June spectrographic observations to reduce the sky contribution and avoid contamination of the image being observed with light from the other image, the data are not reliable for absolute spectrophotometry. The March data show that image B was definitely fainter than image A and are consistent with the result of Young *et al.*⁴ that A was ~ 0.3 mag brighter than B. The 9 June data indicate that the two images were approximately equal in brightness. The data obtained in March are suitable for measurements of the equivalent widths of the C IV $\lambda 1,549$ and C III] $\lambda 1,909$ emission lines. We did not observe the UV spectrum on 9 June but can derive equivalent widths for the C III] and Mg II $\lambda 2,800$ emission lines from those data. The spectra are shown in Fig. 2, and the equivalent widths are given in Table 2. To produce an internally consistent set of equivalent widths for a given line, we used an identical wavelength interval to define the extent of the line profile on all of the individual spectra. Each spectrum is actually derived from two independent spectra. The spectrograph has two entrance apertures, and an object under observation is switched between them, ultimately spending equal time in both. The data from the two apertures are reduced separately. Comparisons of the equivalent widths determined from the independent spectra and similar data for other objects indicate that the errors in the equivalent widths are unlikely to exceed $5 \text{ \AA}$. Although we include values from earlier workers, we have not compared their values with ours because of the possibility of systematic differences between groups in methods of evaluating equivalent widths in data of only moderate signal-to-noise ratio. In our March data, the equivalent widths of C IV and C III] in images A and B agreed to within the measuring errors, as the earlier workers found. However, the June data show a marked change, as C III] and Mg II have significantly smaller equivalent widths in

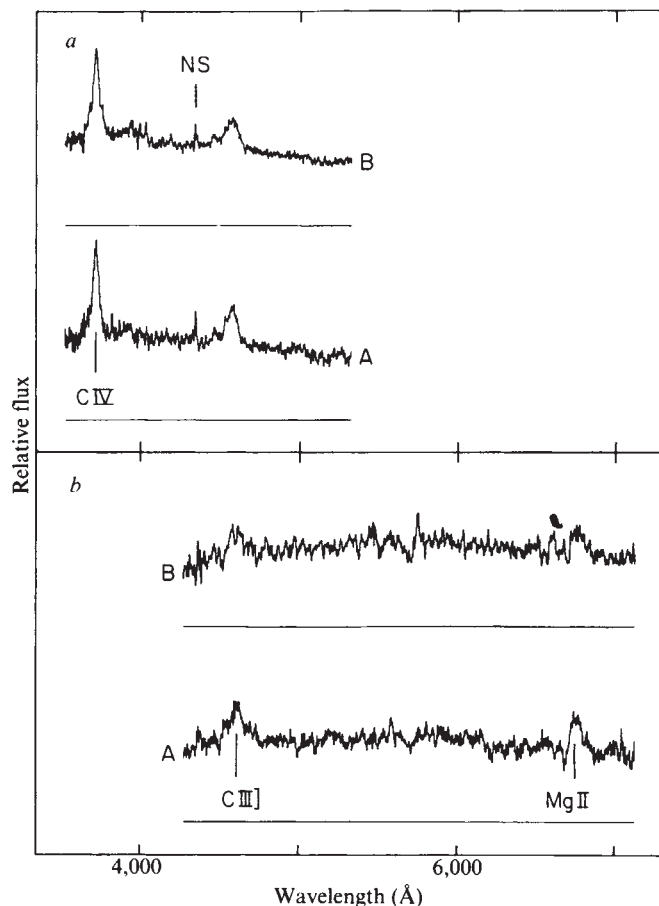


Fig. 2 Spectra of the double quasar. *a*, 12–14 March 1980; *b*, 9 June 1980. The total observing time for each of the March spectra was ~ 2.5 h, but observing time limitations allowed only 32 min for each in June, resulting in noisier spectra from the June data. A and B had very similar spectra in March, but in the June spectra the equivalent widths of the emission lines are smaller for B than for A. NS marks the position of a partially uncanceled strong night sky emission line.

B than they do in A. Also, for both objects, the June equivalent widths of C III] differ from those measured from the March data, though the change for image A is only of marginal significance given the estimated errors. A simple interpretation of these data is that between March and June, the continuum of A faded slightly, the continuum of B brightened substantially, and all emission lines remained constant in flux. This spectroscopically motivated conclusion is in complete accord with the photographic photometry mentioned above. Because no change in the properties of the gravitational lens would be expected to produce changes in the equivalent widths of various lines, we conclude that 0957+561 is an intrinsically variable quasar, and the spectral differences between the two images in June result from different light-travel times for the rays responsible for each image. Other studies of variable QSOs (see for example, refs 7, 8) indicate that, at least on short time scales (≤ 1 –10 yr), only continua have been observed to vary while line fluxes remained

Table 2 Equivalent widths ($\text{\AA}$) in the observed frame

	0957+561 A			0957+561 B		
	C IV $\lambda 1,549$	C III] $\lambda 1,909$	Mg II $\lambda 2,800$	C IV $\lambda 1,549$	C III] $\lambda 1,909$	Mg II $\lambda 2,800$
12, 13 March	60	47	—	55	48	—
9 June	—	58	41	—	32	28
WCW ¹	68	54	28:	70	55	Present
WW ³	—	58	61	—	58	62
Young <i>et al.</i> ⁴	77	62	58	74	58	45

constant. Furthermore, the ratio of equivalent widths derived from the June data of C III] for the two images is different in size from that of Mg II, but this difference is consistent with a considerably greater galaxy contribution to the continuum of B in the spectral region of Mg II than in the region of C III], as was pointed out by Young *et al.*⁴

The double quasar 0957 + 561 A, B is, therefore, variable in both brightness and spectrum. The light variations were accompanied by corresponding changes in the equivalent widths of emission lines, and we conclude that the quasar is an intrinsic variable with behaviour similar to that observed in other variable quasars. Establishing a value for a constant time-difference between variations of the two images would not only confirm the gravitational lens hypothesis, but would add an additional parameter to models for the lens. As Young *et al.*⁴ have pointed out, it is not even clear which image is formed by the path of shortest light-travel time.

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An outburst of comet Tempel-2 observed spectrophotometrically

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Periodic comet Tempel-2, first discovered in 1873, is a possible target of a spacecraft flyby in the 1980s. Accurate physical measurements of this comet have only recently been attempted. Few spectrophotometric data exist for any faint comet, but we present here such data for Tempel-2 at a heliocentric distance of 2.97 AU. The data were obtained during an outburst in which the comet exhibited heightened CN and C₂ emission over a quiescent phase. Comet flares of such magnitude are exceptional, and are particularly surprising for a periodic comet at such a large heliocentric distance.

We observed Tempel-2 on 22.2 December 1978 UT using the Oke multichannel spectrometer¹ and 200-inch telescope. Fluxes of the comet and the sky 40 arc-s away were measured simultaneously in 30 channels in the wavelength range 3,200–10,500 Å. The bandwidth was 160 Å below 5,720 Å and 360 Å above (in rectangular bandpasses). All measurements were made using a 14-arc s aperture. The air mass of the comet was 1.1 during the observations.

Tempel-2 was post-perihelion at a heliocentric distance of 2.97 AU with a phase angle of 8.61° and a magnitude at 5,600 Å of ~18.5. Identification at the telescope was confirmed by motion of the comet on the television monitor with the expected rate and direction. No coma was evident on the monitor, but the seeing was poor.

The sky background was removed by differencing the count rates in the spectrometer apertures on the comet and the sky. The relative sensitivity of the apertures was determined by observing the same object in each aperture. The spectral reflectance was obtained by taking the ratio, in each channel, of the flux due to the comet and that of HD 28992, a G1 V star in the Hyades observed at nearly the same air mass. (A Hyades star was chosen because it would be expected to have a nearly solar metal abundance and thus provide the closest possible spectral match to the Sun.)

The accuracy of this method was confirmed by applying it to measurements of the bright asteroid 14 Irene. An excellent match with the data of Chapman and Gaffey² was obtained within the confidence limits of their data (±3%). The spectral reflectance data for the comet are listed in Table 1 and plotted in Fig. 1, including channels from 3,480 Å to 8,160 Å. Channels at longer and shorter wavelengths are very noisy and are not shown.

Comparison of our data with the photometry of other observers (ref. 3 and R. L. Millis, personal communication) shows that our observations were made during an outburst in which the continuum brightness of this comet temporarily increased by about one visual magnitude. This suggests that the reflectance spectrum is produced principally by scattering of sunlight off particles around the nucleus and is not diagnostic of the nucleus itself. An additional outburst of ~1.5 mag was observed by Zellner *et al.*⁴ a month later with broadband photometry. Although one flare⁵ of Tempel-2 was reported during its 1946 apparition, to our knowledge these are the first confirmed outbursts of Tempel-2.

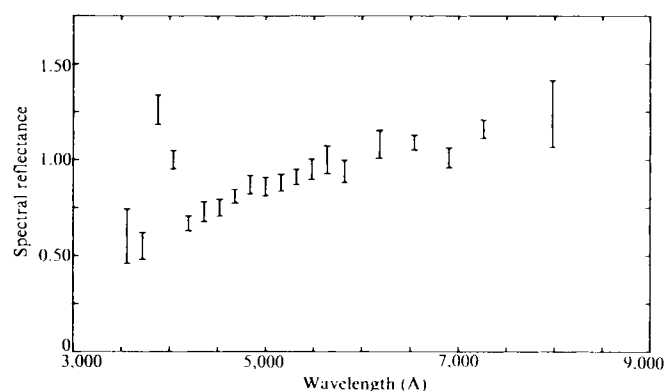


Fig. 1 The spectral reflectance of Tempel-2, normalized to unity at 5,640 Å. These measurements were made on 22.2 December 1978 UT when the comet was post-perihelion at a heliocentric distance of 2.97 AU. The data are indicated with $\pm 1\sigma$ confidence intervals.

The occurrence of a series of outbursts at such a large heliocentric distance is surprising. Only one other short-period comet, Schwassman–Wachmann 1 (the heliocentric distance of which varies between 5.5 and 7.3 AU), has been reported to exhibit high-amplitude outbursts at heliocentric distances ≥ 3 AU. Note that Schwassman–Wachmann 1 is not a typical short-period comet for several reasons⁶. First, this comet undergoes frequent non-periodic variations in its brightness by a factor of 100. Second, there has been no confirmed measurement of molecular emission from Schwassman–Wachmann 1 other than a weak CO⁺ feature^{7,8}. Observations of a 1–2 mag

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flare in any comet are quite rare, even near perihelion where such effects would be expected to be observed most readily, due to both the increased brightness and expected increase in activity of comets at perihelion. Richter⁹ has looked for anomalous outbursts of every comet observed photometrically between 1880 and 1947. Of these 358 comets, only 12 had observed flare-ups in total light of a magnitude comparable with that which we measured for Tempel-2 (~1 mag).

With the measured spectral reflectance of Tempel-2, we can estimate the abundance of gas and dust in the coma and the albedo of the dust. The emission bands in the channels at 3,880 Å and 4,040 Å are due to the $\Delta\nu=0$ band of CN at 3,883 Å and the 4,050 Å C_3 group and permit a determination of the CN and C_3 production rates. The CN flux is taken to be the excess flux, in the channel centred at 3,880 Å, above the continuum represented by a linear interpolation between the spectral reflectances in the adjacent channels at 3,720 Å and 4,200 Å. Using the flux (J. B. Oke, personal communication) of the standard star HD84937, we find that this excess represents a flux in the CN band of $8.8 \times 10^{-18} \text{ W m}^{-2}$. This flux is next converted to a number abundance of CN molecules using the relation between the flux per unit column-number density and the heliocentric Doppler velocity as reported by Tatum and Gillespie¹⁰. For the geocentric distance of 2.062 AU and an r of 9.8 km s^{-1} , the CN abundance in the field of view is 1.6×10^{27} molecules.

In the Haser model^{11,12}, observed molecular column densities in the steady state case can be related to production rates by

$$D(R) = C[\exp(-R/l_d) - \exp(-R/l_p)]/R^2 \quad (1)$$

where $D(R)$ is the number density of a given molecule at a distance R from the nucleus, l_p is the scale length for the decomposition of the parent molecule, l_d is the scale length for the destruction of the observed species, and C is a constant of proportionality. Using the scale lengths given for CN by Delsemme¹³ and the CN abundance derived above, we find a production rate of 5×10^{23} CN molecules s^{-1} . It is ~100 times greater than the upper limit of 6×10^{21} CN molecules s^{-1} found by Spinrad *et al.*³ during a post-perihelion quiescent phase two months earlier at a heliocentric distance of 2.66 AU. Similarly we find a C_3 production rate¹² of $\sim 10^{24}$ C_3 molecules s^{-1} . Note that although the region of sampled sky was less than l_d from the comet nucleus, the R^2 term in equation (1) limits the amount of emission to be expected in the sky channel to $\leq 10\%$ of

the emission in the object aperture, if the Haser model is applicable.

Next we investigate the role of dust in contributing to the brightness of the comet through reflection. Following Spinrad *et al.*³ we assume a CN/ H_2O number ratio of 1:200 and a dust/gas mass ratio near unity. We then have a dust production rate of $3,000 \text{ g s}^{-1}$. Delsemme and Miller¹⁴ derive a relationship between the total gas vaporization rate of a comet and its dust expansion velocity. Arbitrarily assuming dust expansion in a 60° cone, one finds a dust expansion velocity $\sim 10 \text{ m s}^{-1}$ (ref. 3). With a dust particle size distribution characterized by a geometric cross-section of $3.17 \times 10^{-7} \text{ km}^2 \text{ g}^{-1}$ (ref. 15) and the dust production rate given above, we derive a total cross-section of $1.6 \times 10^3 \text{ km}^2$ for the dust situated within the projected boundary of our aperture.

Finally, we can estimate the albedo of the dust by assuming that all the continuum flux is produced by scattering of sunlight off of the dust. Using the cross-section obtained above and a nominal phase coefficient of $0.03 \text{ mag deg}^{-1}$ (ref. 3), a geometric albedo of $\sim 6 \times 10^{-4}$ would be required at 5,600 Å. This contrasts with the geometric albedo of 0.10–0.15 suggested by Spinrad *et al.*³ for the nucleus. The albedo we derive is actually a lower limit. Assuming a 'burst' model, where all of the molecules and dust contributing to the comet brightness are located inside the projected object aperture, the CN abundance, as before, is 1.6×10^{27} molecules. Again assuming a CN/ H_2O number ratio of 1:200 and a dust/gas mass ratio near unity, we find $9.2 \times 10^6 \text{ g}$, or 2.9 km^2 , of dust within the field of view. This leads us to a geometric albedo ~ 0.3 , in closer agreement to the value obtained by Spinrad *et al.*, and nearer to what one would expect for ice grains.

The burst model requires that the time, t , elapsed since the outburst of matter from the nucleus is less than r/v , where r is the maximum radial distance from the nucleus still within the aperture and v is the thermal velocity of the parent molecule of CN. With a v of 0.71 km s^{-1} (ref. 16) and an r of $1.05 \times 10^4 \text{ km}$, we find that $t \leq 10^4 \text{ s}$. Since the albedo of 0.3 derived from this model certainly seems to agree within a factor of 10 with what one would expect, these results suggest that this observed outburst of Tempel-2 was of relatively short duration, ≤ 1 day. Such rapid outbursts have in fact been observed in Schwassman-Wachmann 1 and seem to occur in the other comets which have exhibited large brightness fluctuations⁹.

The present results suggest rapid variability of Tempel-2. More quantitative results on faint comets are hampered by the extreme paucity of data. In particular, more spectrophotometry of faint comets is needed to constrain comet modelling.

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* Unreliable channel.

Stability of zeolites under electron irradiation and imaging of heavy cations in silicates

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The aluminosilicate framework of synthetic 'zeolites' of type A (Na^+ -form) and ZSM-5, as well as the Na^+ -forms of faujasite (Fig. 1) in their so-called X- and Y-variants (fuller structural descriptions are given in refs 1 and 2) have recently been imaged at near-atomic scale by high-resolution electron microscopy (HREM)³⁻⁵. However, the as-prepared synthetic and naturally occurring zeolites, all of which contain alkali metal and alkaline-earth exchangeable cations and much constitutional, intracrystallite water, are converted within minutes into an amorphous state on examination in an electron beam of the intensity required to yield high-resolution images. The specimen has to withstand 10^6 electrons $\text{\AA}^{-2}$ bombardment within a few minutes. To extract further information from HREM about the local atomic structure, and, in particular, to identify the siting of the various cations that may be accommodated in these catalytically important and shape-selective materials, methods have to be developed which retain the structural integrity of the zeolite for a reasonable time under electron irradiation *in vacuo*. It has been shown³ that dehydration of the zeolite before observation significantly increases its lifetime in an electron beam. Here we show that the commercially important⁴ zeolite-Y may be chemically stabilized against electron irradiation damage by the simple expedient of incorporating uranyl (UO_2^{2+}) ions into the structure beforehand. Beam sensitivities are decreased by factors of 10–100 in this way, but appropriate heat-treatment of the UO_2 -exchanged zeolite is required to achieve this stability and hence to locate these heavy cations.

For the present study the UO_2^{2+} ion has two advantages. First, its propensity to form strong links with surrounding oxygen ligands: this along with the space-filling nature of this bulky ion should strengthen and rigidify the relatively fragile aluminosilicate framework of the zeolite. Second, the U atom ($Z = 92$) is a strong scatterer of electrons which should make it relatively easy to locate the centres of the linear UO_2^{2+} ions in high-resolution images.

Low-dose electron diffraction studies showed that straightforward cation-exchange of Na^+ by UO_2^{2+} in Na-Y and drying at $\sim 90^\circ\text{C}$ does not significantly change the lifetime of the zeolite. Subsequent high-magnification imaging results in rather rapid amorphisation, as found previously for Na-A, -X and -Y. Simultaneously with this transformation to a zeolitic glass we observe (Fig. 2) growth of a thin surface film. Both the image, which was recorded for the optimum objective lens defocus condition ($\Delta f = -652 \text{ \AA}$) for a lens having spherical aberration coefficient $C_s = 1.2 \text{ mm}$, and the corresponding optical transform (inset Fig. 2) show that interatomic spacings of 2.5–2.7 $\text{\AA}$ predominate. A survey of the various phases of uranium oxide and of uranium metal reveals that U–U separations, when projected normal to common low-index zone axes, should cluster around 3.5 $\text{\AA}$ for phases of the crystalline UO_3 -type, around 2.2–3.3 $\text{\AA}$ for UO_2 and 2.5–2.9 $\text{\AA}$ for α -uranium metal. Clearly the surface film, the identity of which has not yet been fully characterized, shows a microcrystalline texture with short-range order extending over only 9–15 $\text{\AA}$.

If, instead of the drying at $\sim 90^\circ\text{C}$, the UO_2^{2+} -exchanged zeolite is heated for extended periods *in vacuo* (typically 15 h at 410°C), the beam resistance of the Y-zeolite is greatly

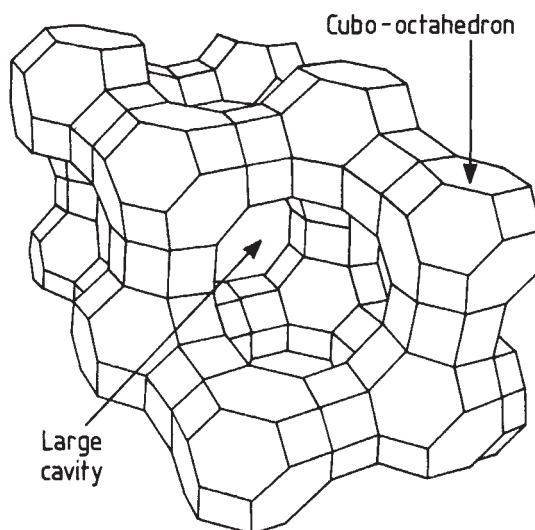


Fig. 1 Faujasite, the parent structure on which zeolites-X and -Y are based (Si:Al ratios differ in X and Y and they differ also from the ratio of 2.48 in idealized faujasite).

enhanced. Thus the micrograph shown in Fig. 3 was obtained after the specimen had been subjected to at least 100 times the exposure previously required to amorphise Na-Y. The pseudo-hexagonal array of dark spots in Fig. 3a (spacings 17 and 16 $\text{\AA}$) shows an essentially one-to-one correspondence with the positions of the 'decorated' super-cages in the [110] projection of zeolite-Y (that is faujasite—see Fig. 3b). Because the image was obtained using the condition of optimum objective lens defocus for structure imaging at $\sim 5 \text{ \AA}$ resolution⁶ we interpret the dark spots as representing the projected charge density of UO_2^{2+} ions occupying the zeolite cages.

This specimen, the IR spectrum^{7,8} of which indicated that the UO_2^{2+} ions were still intact and that the OH frequencies were greatly reduced in intensity after heating to 410°C , could be given short bursts of extremely high electron irradiation (by

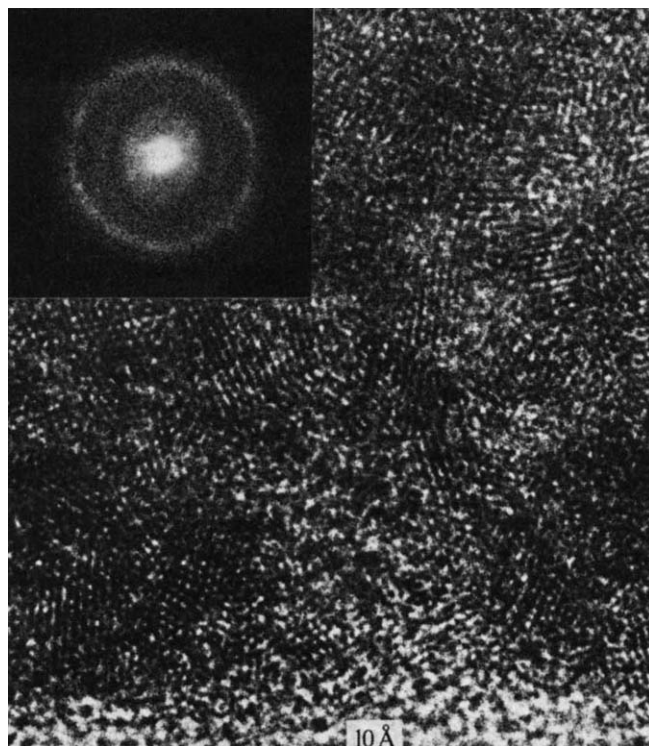


Fig. 2 High-resolution image with optical diffractogram (inset) of microdomain texture produced on the surface of hydrated uranyl-exchanged zeolite-Y on amorphisation in a 200-keV electron beam (see text).

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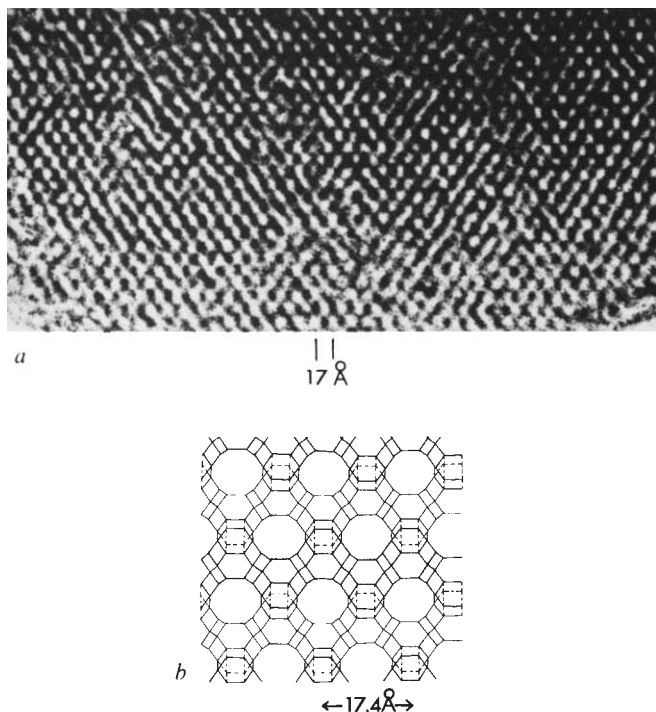


Fig. 3 *a*, High-resolution image along [110] showing location of uranyl ions inside a dehydrated uranyl-exchanged zeolite-Y. This crystal survived a dose of ≈ 100 times that experienced by the specimen shown in Fig. 2. *b*, Schematized projection, along [110], of faujasite (zeolite-Y) showing that the large cavities are 17.4 and 15.1 Å apart. The dark spots in *a* are, therefore, identified as the cavities decorated with UO_2^{2+} ions (see text).

removing condenser apertures and focusing the illumination) without any apparent change in structural integrity. Excessive beam currents did, however, lead to the growth of electron-opaque spherical crystallites (~ 300 Å diameter) within a matrix of amorphized zeolite. This confirms that the Y-zeolite contained, as suggested by Fig. 3*a*, an initially homogeneous distribution of incorporated UO_2^{2+} ions. The mobility of the specimen, when subjected to excessive beam currents, suggests that the temperatures reach well beyond 1,000 °C.

Although the precise environment of the UO_2^{2+} ion after high-temperature treatment (410 °C) and hence the detailed reasons for the enhanced beam stability are not yet fully identified, it is plausible to suppose that residual water has a key role (see also ref. 1). If water molecules are present in appreciable quantities (either as hydration shells or bound to cage walls) a ready mechanism exists for the exsolution of the UO_2^{2+} ions. Thus, at ~ 90 °C, the substitution of $2\text{H}_3\text{O}^+$ for UO_2^{2+} would be possible. At 410 °C, as the IR results reveal, this substitution cannot occur: the UO_2^{2+} ions are firmly chemically bound to, and thereby strengthen, the aluminosilicate framework.

The possibility of using UO_2^{2+} ions to decorate or label cages, cavities, tunnels and interlamellar spaces in other crystalline or non-crystalline aluminosilicates, as well as of charting the connectivity of the voids in, and the diffusion of heavy ions through, such materials is being investigated.

There is considerable technological interest in the stability and environment adopted by uranium in various host matrices. This stems from the need to sequester actinide waste products effectively in environments that prevent migration of the radioactive ions into the biosphere. During this work, we have incidentally discovered that both uranium glasses and uranyl ions embedded in 'dehydrated' zeolites are remarkably stable to quite intense 200 keV electron bombardment.

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Photochemical cleavage of water by photocatalysis

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A bifunctional redox catalyst, composed of Pt and RuO_2 co-deposited on a colloidal TiO_2 carrier, is a highly potent mediator for water decomposition by visible light¹. The system contains apart from the sensitizer ($\text{Ru}(\text{bipy})_3^{2+}$) an electron relay—methylviologen. The latter is reduced on light excitation, and the photoreaction is coupled with catalytic steps² generating H_2 and O_2 from water. To rationalize the surprisingly high efficiency of this photoredox system, we proposed a mechanism involving species adsorbed at the TiO_2 surface. This led us to explore sensitizers which through suitable functionalization show an enhanced affinity for adsorption at the particle–water interface. We describe here the performance of electron relay-free systems capable of efficiently decomposing water into H_2 and O_2 under visible light illumination. A bifunctional redox catalyst composed of RuO_2 and Pt co-supported on colloidal TiO_2 particles is used. The only other component present is a sensitizer. Amphiphilic surfactant derivatives of $\text{Ru}(\text{bipy})_3^{2+}$ exhibit extremely high activity in promoting the water cleavage process. Adsorption of the sensitizer at the TiO_2 particle–water interface and electron ejection into the TiO_2 conduction band are evoked to explain the observations. Exposure to UV radiation leads to efficient water cleavage in the absence of sensitizer.

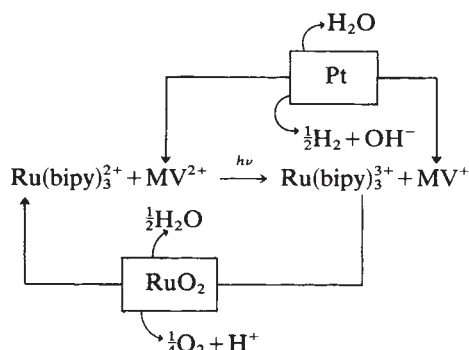
The successful operation of this device depends on the proper preparation of the catalyst. In our experiments 100 mg of H_2PtCl_6 was added to 250 ml of water and stirred for 30 min without heating. 85 ml of a 1% sodium citrate solution was added and the mixture refluxed for several hours until the colour changed from yellow to brown. At this time the characteristic absorption of PtCl_6^{2-} has disappeared, the spectral features in the visible and UV being typical of those of an ultrafine Pt sol³. The absorbance at 450 nm is 0.55 (pathlength 1 cm). After cooling, the solution was stirred with Amberlite MB 3 exchange resin to remove excess citrate until the conductivity decreased below $5 \mu\text{S cm}^{-1}$. The solution was filtered through a 0.45 μm Millipore filter and a clear liquid was obtained with an absorbance of 0.50 at 450 nm. 12 mg of TiO_2 was added to 12 ml of this solution and exposed for several minutes to ultrasound in a water bath.

The TiO_2 particles have an anatase structure and were prepared from TiOSO_4 by acid hydrolysis. The surface area is 200–240 m^2 per g and the hydrodynamic radius of the particles 470 Å. They are also doped by 0.1% RuO_2 and by 0.4% Nb_2O_5 .

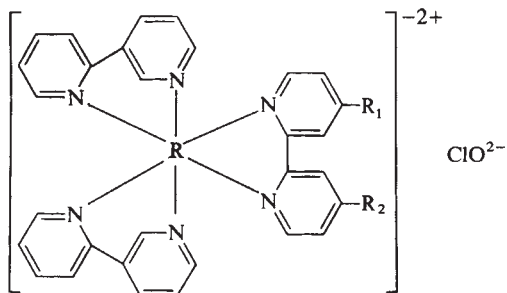
Illuminations were carried out with an XBO-450 W Xe lamp fitted with a water jacket to remove IR irradiation. The gas produced was analysed by gas chromatography. A Gow Mac system, carbosieve column (35 °C) and Ar (for H_2) or He (for O_2) carrier gas was used. The volume of the solution was invariably 24 ml and contained 500 mg l^{-1} TiO_2 as well as 40 mg l^{-1} Pt; it was contained in a Pyrex flask fitted with a septum. The solution was deaerated before illumination by flushing with Ar or He.

The colloidal Pt/TiO₂/RuO₂ particles were first irradiated without the sensitizer. If no cutoff filter is used to remove UV irradiation (in this case transmission is restricted by the Pyrex flask) production of H₂ and O₂ is observed through direct band gap excitation of the semiconductor particles. After 10 h exposure to light 3.4 ml of H₂ was evolved. Oxygen was formed in stoichiometric proportions. Stirring of the solution in the dark for 10–20 h results in H₂/O₂ recombination. This reaction can be avoided by removing the gas mixture after photolysis with a stream of N₂. On renewed exposure to light, the water splitting continues at the initial rate, 0.34 ml H₂ h⁻¹. This cycle can be repeated many times. H₂ and O₂ generation is completely suppressed if a 400-nm cutoff filter is placed in the light beam. These results show that the particles can split water into hydrogen and oxygen after band gap excitation. The yields are surprisingly high showing efficient electron-hole separation. Water splitting on TiO₂ and SrTiO₃ powders has been observed before^{4–9}, but with much lower efficiency.

The Pt/TiO₂/RuO₂ particles can also be used as catalysts for water splitting by visible light in the presence of a suitable sensitizer and methylviologen as an electron relay¹,



The present study shows that this system can be made operative even in the absence of the electron relay. The sensitizers used were ruthenium trisbipyridyl complexes of the structure:



where

- $\text{R}_1 = \text{R}_2 = \text{H}$: Ru(bipy)_3^{2+}
- $\text{R}_1 = \text{R}_2 = n\text{-hexadecyl}$: $\text{Ru(bipy)}_3^{2+} \cdot 2\text{C}_{16}$
- $\text{R}_1 = \text{CH}_3$, $\text{R}_2 = n\text{-octadecyl}$: $\text{Ru(bipy)}_3^{2+} \cdot \text{C}_{18}$
- $\text{R}_1 = \text{CH}_3$, $\text{R}_2 = n\text{-doceyl}$: $\text{Ru(bipy)}_3^{2+} \cdot \text{C}_{12}$

Data from visible light illumination of solutions containing the sensitizer and the bifunctional redox catalyst are displayed in Fig. 1. The amount of hydrogen generated is compared for the four different sensitizers. Oxygen remained in stoichiometric ratio to the H₂ produced. These solutions contained no buffer and were adjusted to a pH of 4.5 by addition of HCl. No pH changes could be detected even after prolonged irradiation. In Fig. 1 the lowest H₂ production rate is observed with the simple Ru(bipy)_3^{2+} complex. Introduction of a long alkyl chain leads to a drastic improvement in the H₂ yields. Of the surfactant derivatives available¹⁰, the mono C-12 substituted complex exhibits optimal efficiency. After a short induction period, the H₂ generation rate was established at 1.5 ml h⁻¹. In view of the small concentration of sensitizer ($\sim 5 \times 10^{-5}$ M), this rate is surprisingly high. At this concentration, light absorption is incomplete even at λ_{max} of the sensitizer. As the solutions are

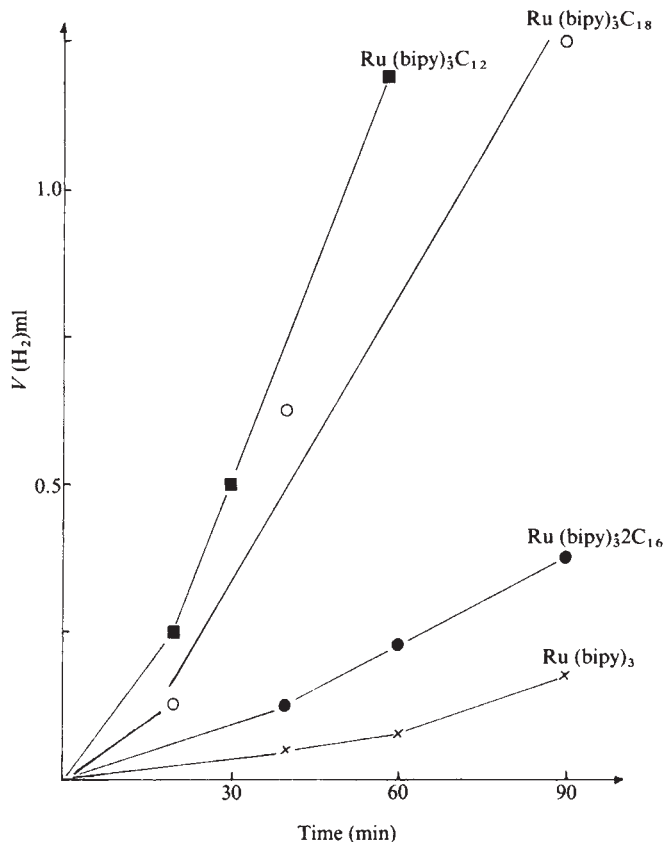


Fig. 1 Hydrogen yields from visible light (>400 nm) photolysis of solutions containing 500 mg per l TiO₂/RuO₂ (0.1%) doped with 40 mg per l Pt, pH = 4.5 adjusted with HCl. Sensitizer concentration:

- $\text{Ru(bipy)}_3^{2+} = 2 \times 10^{-4}$ M;
- $\text{Ru(bipy)}_3^{2+} \cdot 2\text{C}_{16} \}$ $= 7 \times 10^{-5}$ M;
- $\text{Ru(bipy)}_3^{2+} \cdot \text{C}_{18}$
- $\text{Ru(bipy)}_3^{2+} \cdot \text{C}_{12} = 5 \times 10^{-5}$ M.

slightly turbid scattering of light cannot be neglected making the determination of quantum yield difficult. However, from a comparison with a non-regenerating H₂-producing system¹¹ ($\phi(\text{H}_2) = 0.13$) used in identical conditions, the quantum yield of the H₂ production with the C-12 substituted Ru-complex is derived as $\sim 5\%$. This may be further improved by optimization of the sensitizer, catalyst and pH condition.

The H₂ production rate does not decrease even after 50 h of irradiation time, the turnover for the Ru complex being >1,000.

These observations may be rationalized in terms of the scheme shown in Fig. 2. The effect of functionalization of the Ru(bipy)_3^{2+} on the H₂ yields confirms that the photoredox processes involve adsorbed species. Excitation of the sensitizer is followed by charge injection into the conduction band of TiO₂. The electron is readily channelled to Pt sites where H₂ evolution occurs. A small Schottky potential barrier at the Pt/TiO₂ junction may assist the electron movement. The back conversion of oxidized sensitizer into its original form is coupled to oxygen formation through RuO₂ which is an excellent electrocatalyst for water oxidation.

The effects observed when the TiO₂ particles are directly excited by band gap irradiation have been well understood since Honda's¹² work on the cleavage of water by UV-ray irradiation of TiO₂ macroelectrodes. The surprisingly high quantum yields of the UV process show that electron-hole separation in the particles is efficient and probably assisted by local fields.

Cleavage of water is possible by four quanta of visible light in an electron relay-free system containing simply a sensitizer and colloidal redox catalyst. A key to achieve high efficiencies is the

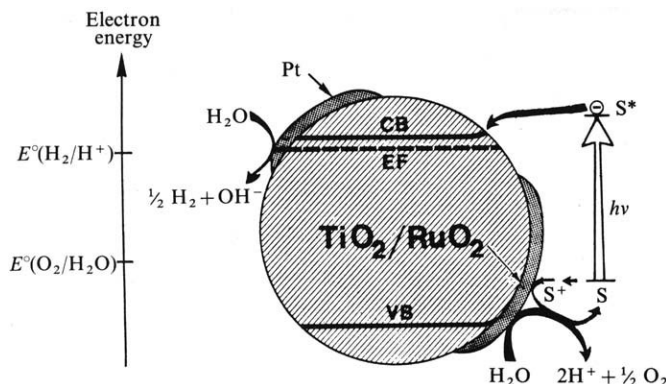


Fig. 2 Processes involved in the photodecomposition of water.

use of surfactant redox chromophores¹³⁻¹⁵ which are adsorbed at the surface of the catalytic particles. The water cleavage on such functional microspheres can thus occur without participation of diffusional processes. These results should encourage research into photochemical conversion of solar energy¹⁶⁻²⁰.

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Crustal influence in the generation of continental flood basalts

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The suggestion that primordial undifferentiated material may exist in the Earth's mantle has recently been revived on the strength of Nd isotope data for two types of young continental rocks—flood basalts¹ and kimberlites². The limited published data show a clustering of Nd isotopic compositions close to those for meteorites with chondritic relative rare-earth (REE) abundance. In contrast, we now present data for samples from the Columbia flood basalt province of the northwestern United States which show large isotopic variability suggestive of mixing processes acting after the separation of the primary magmas from their mantle source.

The Columbia province contains ~200,000 km³ of predominantly basaltic rocks which were fed from fissure eruptions 6–17 Myr ago³. The province is in the volcanically and tectonically active Pacific north-west of the US, to the east of the present locus of Cascade andesitic arc volcanism. This area is the most extensively studied of the major flood basalt provinces³⁻⁵. Five

main stratigraphic units in the basalts have been identified and in rough stratigraphic order, are named: Imnaha, Picture Gorge, Grande Ronde, Wanapum and Saddle Mountains. The field relations and major element composition of these units are detailed elsewhere³.

We have analysed the Nd and Sr isotopic composition of samples from each of the stratigraphic units, and from a series of unclassified Columbia basalts of variable composition which overlie the Grande Ronde in northeastern Oregon⁶ and also mafic lavas from various localities in northern California. Collection localities and major and trace element composition of these samples are given elsewhere⁷. The Sr data measured here augment studies⁵ of the Columbia region, and demonstrate how combined Nd and Sr isotopic analysis aids the understanding of the origin of complex chemical and isotopic variations found in these lavas.

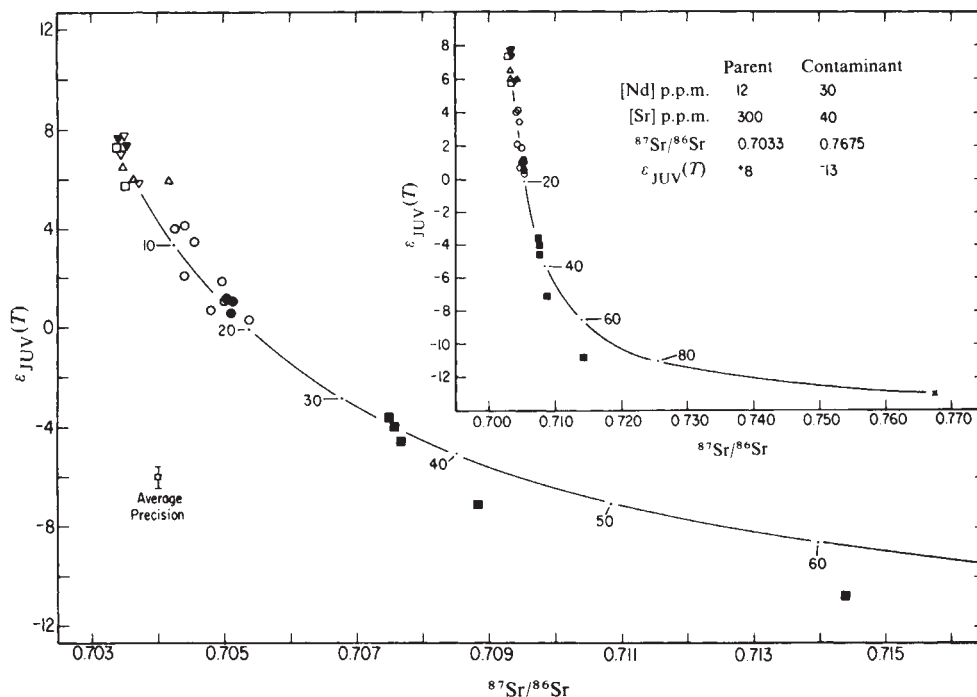
Isotopic results are shown in Fig. 1. Analytical techniques have been described elsewhere^{7,8}. All Nd data are fractionation corrected to ¹⁴⁸NdO/¹⁴⁴NdO = 0.242436 (corresponding to ¹⁴⁶Nd/¹⁴⁴Nd = 0.7219). Sr values are fractionation corrected to ⁸⁶Sr/⁸⁸Sr = 0.1194 and are reported relative to a value of ⁸⁷Sr/⁸⁶Sr = 0.71014 for NBS 987 Sr.

Figure 1 shows the large isotopic variability within the basalts of the Columbia group. Particularly important is the good correlation between Nd and Sr isotopic variation. Note the general correlation between isotopic composition and stratigraphic position as the variability extends in the order, Picture Gorge ($\epsilon_{\text{JUV}} = +7.7$ to $+7.4$), Imnaha (7.8 to 5.9), Grande Ronde (+4.0 to +0.3), Wanapum (+1.2 to +0.6) and Saddle Mountains (−3.6 to −10.8). In particular, the basalts of the Grande Ronde group, which represent the largest volume of the Columbia sequence, exhibit ϵ_{JUV} values ranging from +4.0 to +0.3, which correlates roughly with stratigraphic position within this group. The volume weighted average Nd isotopic composition is thus resolvable displaced to values of $\epsilon_{\text{JUV}} > 0$. However, the high degree of correlation of Nd and Sr isotopic variations suggests that this range may be the result of mixing between at least two components: primary magma with $\epsilon_{\text{JUV}} \geq 8$, ⁸⁷Sr/⁸⁶Sr ≤ 0.7033 , and a source of negative ϵ_{JUV} and high ⁸⁷Sr/⁸⁶Sr. At least two sources of negative ϵ_{JUV} and high ⁸⁷Sr/⁸⁶Sr can be proposed: (1) old, metasomatically enriched, mantle^{9,10}; (2) materials derived from the continental crust underlying the province. Verification of the former possibility is hindered by the lack of knowledge of the degree and time scales over which heterogeneities are formed and maintained in the mantle. Similar Nd and Sr covariation, to that displayed in Fig. 1, have been reported for volcanic rocks from Patagonia¹¹ and Italy¹². In the latter case, the co-variation was interpreted to be the result of mantle heterogeneity rather than crustal contamination primarily due to a positive correlation between Sr concentration and ⁸⁷Sr/⁸⁶Sr in these lavas. This argument cannot be applied to the Columbia basalts because they exhibit, in general, poor correlations between Sr and ⁸⁷Sr/⁸⁶Sr. In the only group that shows good Sr versus ⁸⁷Sr covariation, the Saddle Mountains group, Sr concentration decreases with increasing ⁸⁷Sr/⁸⁶Sr. Support for a chemically and isotopically heterogeneous source for the Columbia lavas comes from the fact that major and trace element compositions of these lavas cannot be simply related by mixing between two end-member components^{4,5}. For example, the Saddle Mountains group contains members which are relatively primitive chemically, and others which are highly fractionated, although these all have $\epsilon_{\text{JUV}} \leq -3.6$ and ⁸⁷Sr/⁸⁶Sr ≥ 0.7075 . However, considering the complexity of magma storage and transport in the upper mantle and crust, it is possible to construct a model which explains much of the elemental and isotopic diversity found for the Columbia lavas, assuming that they were all generated from a single homogeneous source.

The inset in Fig. 1 includes Nd and Sr isotopic data for a xenolith recovered from a flow of the Wanapum group. This xenolith was ~3 cm in diameter and consisted primarily of quartz with minor cristobalite. Its major element composition

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Fig. 1 Nd-Sr isotopic variation of Columbia River and some northern California basalts. Data for a metasedimentary crustal xenolith are included in the inset (×). The line is a calculated mixing curve between end-member compositions given in the inset. Numbers on the curve are the percentage of 'contaminant' in the mixture. The deviation from the curve of the two points with large negative ϵ_{JUV} suggests that these two basalts may have assimilated material with lower $^{143}\text{Nd}/^{144}\text{Nd}$ and/or $^{87}\text{Sr}/^{86}\text{Sr}$ than measured for the xenolith. All data have been corrected for isotopic evolution since the time of eruption. ▼, Picture Gorge; ▽, Imnaha; ○, Grande Ronde; ●, Wanapum; ■, Saddle Mountains; □, NE Oregon; △, North California. ϵ_{JUV} is the relative difference of the samples $^{143}\text{Nd}/^{144}\text{Nd}$ (in parts per 10,000) from that measured for the eucrite Juvinas ($^{143}\text{Nd}/^{144}\text{Nd} = 0.512636$).



and texture suggest a metasedimentary crustal protolith. This xenolith has very high $^{87}\text{Sr}/^{86}\text{Sr}$ (0.768) and negative ϵ_{JUV} . Sm-Nd and Rb-Sr model ages for the xenolith, calculated in the T_{ICE} manner⁸, are 1,320 and 1,420 Myr, respectively. The near agreement of these two ages suggests both that little Rb-Sr or Sm-Nd fractionation occurred during the entrapment of the xenolith in the lava, and that this may represent the formation age of the protolith material. If true, this xenolith may be the first identified example of a Precambrian crustal section existing beneath the Columbia Plateau.

While both the major element and oxygen isotopic composition of this particular xenolith argue against its being the specific crustal end member involved, it can be used to illustrate what effect assimilation of crustal materials would have on the Sr and Nd isotopic composition of the Columbia lavas. For example, Fig. 1 shows a calculated mixing curve between end-member compositions given in the inset. The 'parent' end member has the composition of the primitive lavas of the Picture Gorge sequence. The 'contaminant', with identical Nd and Sr isotopic composition and Sr concentration as the measured xenolith, requires an ~78% higher Nd concentration than found for the xenolith to give the curve in Fig. 1. The calculated curve lies within analytical uncertainty of all but two basalt data points. Similar mixing calculations using a crustal end member with less radiogenic Nd and Sr isotopic composition and a higher Sr/Nd would produce a mixing curve with less inflection than that shown and hence provide a better fit to the basalt data. Although the exact crustal end member is not defined by present data, the coherence of the basalt Nd and Sr isotopic data about a single curve strongly suggests that mixing between primary magma and materials derived from the continental crust played a major part in controlling the isotopic variability of the lavas.

Using the end-member compositions given in Fig. 1, magmas of the Grande Ronde group may have assimilated between 10 and 20% of their Sr and Nd contents from the continental crust. Considerations of the thermal effects of basalt intrusion into the continental crust¹³ also suggest that "basalt sheets at 10 km depth, for example, can develop partially molten zones of country rock equal to 10–20% of their own thickness". This implies that the ϵ_{JUV} values which approach zero for the largest volume of the Columbia group, the Grande Ronde, may reflect a most probable mass balance between primary magma and assimilated crust due to the degree of heat transfer possible from the Grande Ronde lavas to the crust. This hypothesis could also be extrapolated to the other occurrences of continental flood

basalts, but is, of course, subject to the elemental and isotopic composition of the crustal end member which may have been involved in each province.

Although the Nd and Sr isotopic variation of the Columbia lavas can be explained by mixing between two components, major and trace elements do not show good co-variation among the groups defined by the isotopic data. However, when controlled by mixing, the resultant isotopic composition of the mixture is determined only by the mass fractions of the various components. This is not necessarily true for the major and trace element composition of the mixture. If processes such as fractional crystallization and various degrees of mixing between highly fractionated magmas and newly injected, rather primitive, magmas occurred simultaneously with crustal assimilation, major and trace element composition of the resulting magmas would be controlled largely by these processes rather than assimilative mixing. Furthermore, the passage of these lavas through the crust may have had a considerable effect on the chemical composition of the residual materials left behind in the crust. In a volcanic province where eruption occurred at a rather rapid rate over a considerable span of time (11 Myr for Columbia³) magmas might be expected to accumulate preferentially and be transported in paths and storage reservoirs created by previous magma batches. Therefore, later magmas would not encounter the original 'sialic' crust but would have to interact with the more mafic material left behind by previous magma batches. This could account for the very high Fe and Ti and relatively low Si concentrations of the Wanapum group compared with the lavas of the Grande Ronde sequence, although these two groups have overlapping ranges in Nd and Sr isotopic composition.

A qualitative model for both the compositional and isotopic variation in the Columbia basalts assuming that they were derived from a homogeneous source region, would require: (1) different degrees or conditions of partial melting in the mantle; (2) transport and storage of primary magmas in crustal storage reservoirs where assimilation of Precambrian crustal material of varying composition, fractional crystallization in response to assimilation, and mixing between dense, Fe rich, highly fractionated liquids and newly injected, rather low density, unfractionated magmas occurred.

Picture Gorge magmas may have escaped the effect of this crustal contamination by their eruption from vents occurring well to the south-west of the majority of dyke swarms in the Columbia province. This hypothesis requires complex

modelling and a better understanding of what really occurs when a basic magma is injected into the lower continental crust before quantitative modelling is possible. However, the very good correlation between Nd and Sr isotopic variation in these lavas and the demonstration that Precambrian continental crust exists under the province, strongly supports the viability of crustal contamination as an explanation for the chemical characteristics of the Columbia group.

If these lavas were derived from a homogeneous source and later modified by a series of complex geochemical interactions, then the parental mantle of the Columbia lavas had an isotopic composition of $^{87}\text{Sr}/^{86}\text{Sr} \leq 0.7033$ and $\epsilon_{\text{JUV}} \geq +8$. These values are very similar to those measured for interoceanic island arcs and marginal basins of the Western Pacific. Whether these results can be extrapolated to other flood basalt provinces is unknown. However, the suggestion of a primordial (that is, $\epsilon_{\text{JUV}} = 0$) source for the Columbia lavas¹ is clearly in error. The present results for the Columbia group do not exclude or confirm the existence of primordial material in the mantle. Rather they indicate the importance of the physical, chemical, and thermal phenomena which would accompany the transport and eruption of large volumes of mafic lavas, such as those of the Columbia province, through an ~30 km thick section of continental crust in a relatively short period of time.

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U series and amino acid dates from Jersey

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Understanding of the raised beach and other interglacial sequences in the southern part of the British Isles has been improved through use of the amino acid racemization technique. This method has permitted correlation of these isolated and fragmentary marine deposits^{1,2} which, except for the Portland site, are located around the shores of the Bristol Channel¹. By applying this technique with the U-series method, we have obtained a geochronometric date from the '8-m' raised beach of the Belle Hougue Cave, Jersey, Channel Islands. Our results suggest that the 8-m raised beach of the Channel should be correlated with isotope stage 5, and that raised beaches above this altitude in the area may pre-date this stage, and thus also the Ipswichian interglacial which forms part of it.

Although there are many raised beaches around the coasts of the western English Channel^{3–5} the non-calcareous nature of the

bedrock geology of the area means that few of these are fossiliferous. The raised beach of the Belle Hougue Cave, although on a base of rhyolitic agglomerate of the Jersey Volcanic series, is strongly cemented by interstitial calcium carbonate and in places contains discrete travertine layers. In the matrix of the beach are the remains of nine species of Mollusca and mammalian bones⁶. Specimens of *Patella vulgata* Linné and fragments of travertine were collected from the beach for analysis (Fig. 2), the shells for the determination of amino acid racemization ratios and the travertine for U-series dating. The resulting D-allo:L-iso ratios and $^{230}\text{Th}/^{234}\text{U}$ dates place an absolute age on the Belle Hougue beach and provide reasonable grounds for correlation with other more northern raised beach sites.

The Belle Hougue Cave lies on the north coast of Jersey (Fig. 1). The beach gravel occurs ~25 m in from the cave entrance and has a height range between 4 m and 8 m above mean sea level (Fig. 2). The gravel contains abundant molluscan remains. Three shells of *P. vulgata* were collected from the main shell concentration in the beach which lies between 30 and 60 cm below the top of the deposit (Fig. 2). The fragmented shells were heavily cemented by CaCO_3 and required careful cleaning before being prepared and analysed for amino acid ratios². The total ratio of D-alloisoleucine to L-isoleucine is given in Table 1. All of the analyses are similar giving a mean D-allo:L-iso value of 0.12 ± 0.02 , except AAL-1219b which has a significantly higher value. This anomalous value may be related to the high degree of cementation of this sample.

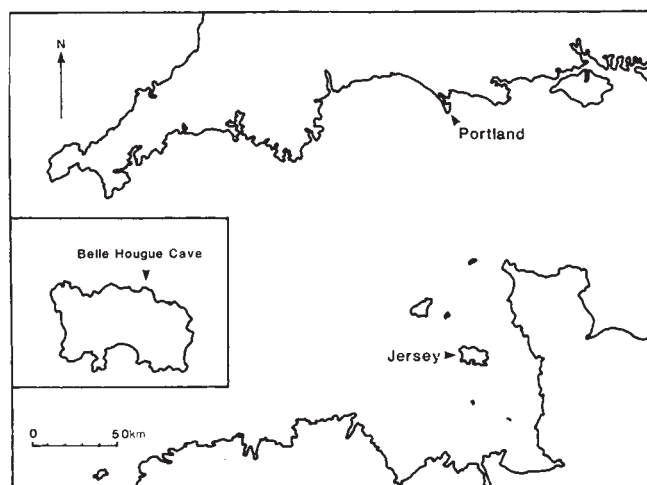


Fig. 1 Location diagram of the western Channel showing the positions of Jersey and Portland. Inset, the site of the Belle Hougue Cave.

The travertine was collected from a layer largely free of pebbles and shell, ~20 cm above the mollusc sample point (Fig. 2). A sample of 200 g was collected and subsplit for duplicate U-series analysis following the technique of Harmon *et al.*⁷. Analytical data and the U-series age of the sample are given in Table 1. The age of ~121 kyr BP indicated by the two runs has a slightly greater than normal uncertainty because of the presence of some detrital Th, and the fact that Th yields were low (<10%) due to the slightly contaminated nature of the travertine. The $^{234}\text{U}/^{238}\text{U}$ ratio, although perhaps rather high, is typical of the ratios obtained from secondary carbonates. Nevertheless, an age within isotopic stage 5, the last interglacial in the marine oxygen-isotopic record⁸, is clear and a stage 5e correlation is suggested.

A stage 5e age for the beach is supported on palaeontological grounds. One of the species of Mollusca found in the beach gravel is *Astrarium rugosum* (Linné) which has a present northern limit near La Rochelle, some 340 km south of Jersey. The occurrence of *A. rugosum* probably indicates a sea surface temperature for the Belle Hougue beach of 3–4 °C above that of the present temperature of the English Channel, a value

Table 1 Amino acid racemization analysis

Sample no.	D-allo:L-iso (combined)	
AAL-1219 a	0.114	
b	0.17	
c	0.112	
d	0.13	
e	0.106	
f	0.136	
Mean of six samples	0.123 ± 0.024	

U-series analysis				
Sample no.	U(conc) (p.p.m.)	$^{230}\text{Th}/^{234}\text{U}$	$^{234}\text{U}/^{238}\text{U}$	$^{230}\text{Th}/^{232}\text{Th}$
79050-1/2	0.46	0.69 ± 0.04	1.56 ± 0.02	16
		Age (kyr BP) 121 ± 14		
		-12		

compatible with that expected from the higher temperatures generally suggested for the last interglacial in both marine⁸ and terrestrial records⁹.

Although a broad stage 5e age for the Belle Hougue raised beach is clear, in terms of wider correlations, several problems occur. The closest agreement of the amino acid values with those noted by Andrews *et al.*¹ is with their group 2 sites at Portland and in the Burtle Beds, which give amino acid values between 0.11 and 0.15. However, these sites both relate to sea levels about 15 m above OD^{10,11} and Portland contains a fauna rather cooler in aspect than that of the present Channel¹². On faunal grounds, therefore, correlation from Belle Hougue to these sites

further north is not exact. Slightly different post-depositional environmental conditions may have occurred at Belle Hougue giving higher amino acid values than would be the case further north where lower temperatures prevailed for the period after the abandonment of the raised beaches. The present mean annual temperature at Portland is 10.7 °C, while in Jersey (St Helier) it is 11.9 °C. A temperature difference of at least this magnitude has probably occurred throughout the past 100 kyr.

Despite the problem of the correlation of the amino acid and sea levels, the absolute age for a raised beach at ~7.5 m above mean sea level supports Bowen's contention¹³ that beaches at this general level in western Channel are of Ipswichian (stage 5) age, and not dated to an earlier interglacial as suggested by Stephens¹⁴. Outside the Channel similar sea level stands ~5–10 m above present sea level have been dated by $^{230}\text{Th}/^{234}\text{U}$ to ~120 kyr.

In Mallorca, Butzer's¹⁵ cycle YI is dated to 120 kyr and has a sea level between 9 and 15 m above the present. In Barbados, Mesolella *et al.*¹⁶ note a sea level 'above the present' at 125 kyr. In New Guinea, the Complex VIIb of Bloom *et al.*¹⁷ has a sea level of +6 m at 125 kyr, and in the stable carbonate platform of Bermuda and in the Bahamas, Harmon *et al.*¹⁸ and Neuman and Moore¹⁹ recognize a +4 to +6 m level stand at 125 kyr. All these results are comparable to the Belle Hougue data in both timing and elevation of the last interglacial sea level maximum, thus allowing correlation of the 8-m raised beach of the western Channel with a similar sea level stand in the Mediterranean, Atlantic and Pacific. This correlation and radiometric date then suggests that the beaches of Andrews *et al.*'s¹ stage 2, and

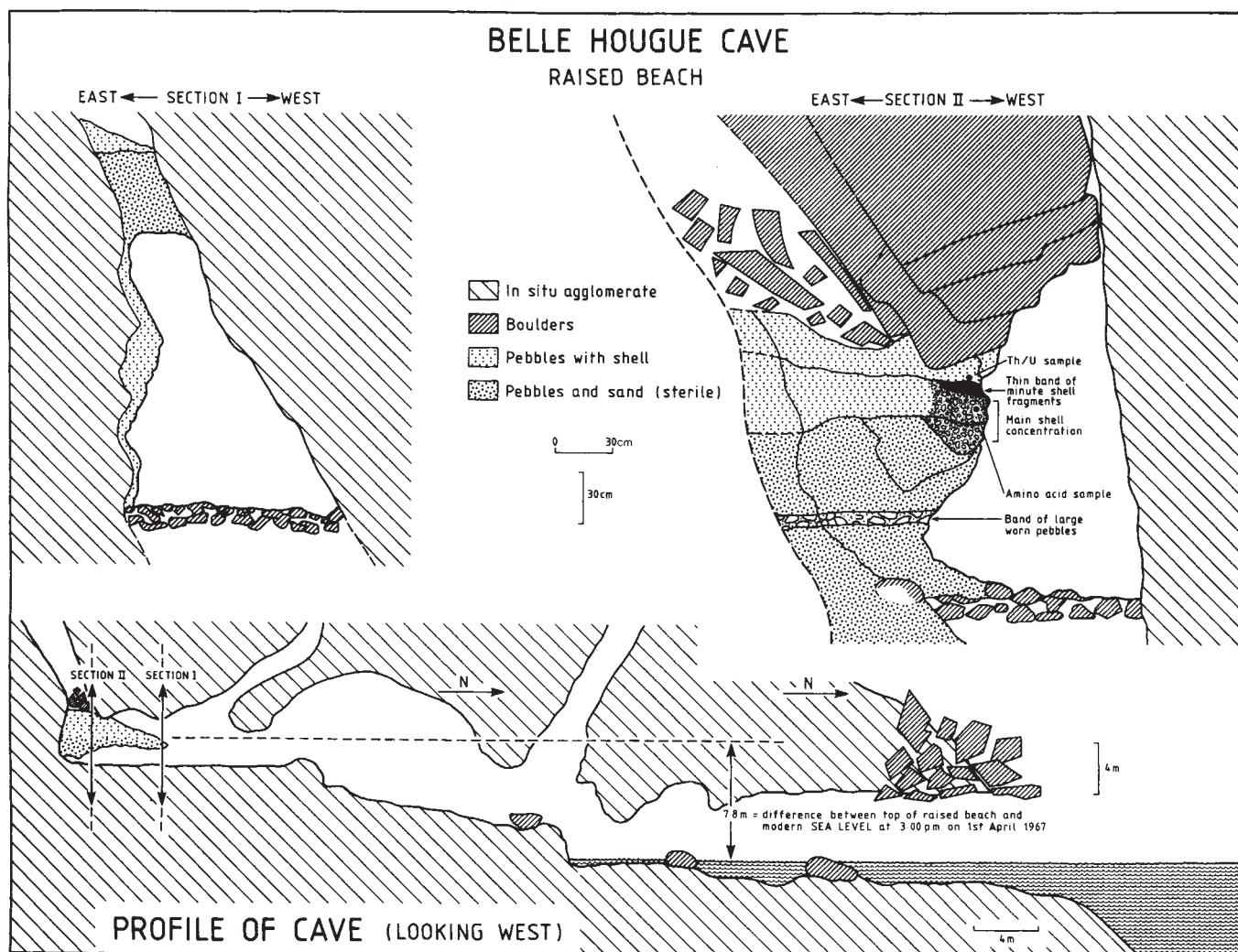


Fig. 2 Sections of the Belle Hougue Cave (based on a survey by J. Campbell, University of Cambridge, Department of Archaeology, 1967).

beaches occupying a similar altimetric range in the Channel Islands³ may be pre-Ipswichian (stage 5) date.

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Relative departures in July temperatures in northern Canada for the past 6,000 yr

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There has been concern about recent temperature trends and the future effects of CO₂ concentrations in the atmosphere^{1,2}; but instrumental records only cover a few decades to a few centuries and it is essential that proxy data sources, such as pollen spectra from peats and lake sediments, be carefully interpreted as climate records. Several workers have shown statistically significant associations between the modern pollen rain and climatic parameters, an approach that by-passes the recognition of pollen/vegetation units. Statistically defined equations that associate abiotic and biotic elements are called transfer functions. We report here on the application of transfer function equations to nine middle and late Holocene peat and lake sediment sequences from northern Canada (Fig. 1).

Two transfer function methods are used³; the first follows the work of Imbrie and Kipp⁴, whereas the second uses multiple stepwise regression to associate present climatic estimates with pollen percentages^{5–9} directly. Modern pollen spectra from two transects are used; one trends SW–NE from south of the treeline in Keewatin to Clyde River, Baffin Island (ref. 9 and H. Nichols unpublished data) (KWA transect), whereas the other is oriented N–S from Fort Chimo in Labrador to Clyde River (ECA transect) (Fig. 1)^{10,11}. Along the KWA transect, Nichols collected ≥ 10 replicate mosses at 29 sites. The ECA transect consists of 87 surface samples from five major collecting localities (Fig. 1)^{10,11}.

Average July temperatures vary between 13 °C and 4 °C along the transects. Pollen data were regressed against this parameter. Pollen percentages were initially computed for all taxa. Many taxa were rare, hence percentages were recalculated on the basis of 13 common taxa for the Keewatin and for 19 taxa for the eastern Canadian Arctic transects. Thus, only common pollen types were used in the transfer function equations. Relevant data are listed in Table 1. We have compared the results from the Imbrie–Kipp and multiple regression approaches and shown that the two methods yield comparable results^{6,8,9}. Equation MKWA–1B is based on the mean pollen percentages at the 29 sites, whereas MKWA–1 is calculated on 293 individual samples. All results indicate that mean July temperatures may be estimated on the basis of only a few taxa. Standard errors on the residuals are $\leq \pm 1$ °C (Table 1).

We applied our transfer function equations to a series of sites (1) to compare broad temperature trends; and (2) to recognize and correlate high frequency events. All sites that we use have been described previously^{12–14}. Nichols¹² interpreted the diagrams from Ennadai, Thompson Landing and the two sites at Coppermine (Fig. 1) in terms of fluctuations in treeline and average July temperature. Transfer function equations MECA and MKWA were applied to lake/peat sites from Labrjor/Baffin Island and Keewatin, respectively. The resulting temperature estimates were smoothed. Finally, relative departures of July temperature were computed for each time-series as: $(x - \bar{x})/s$, where $\bar{x}$ and s are the mean and standard deviations (Table 2), respectively. Oscillations in temperature estimates could be simply 'noise'; thus, we examined residuals from time/temperature linear correlation models by the Runs Test, the null hypothesis was rejected in all cases but Thompson Landing. These results imply that the runs of temperature departures on Fig. 2 are not spurious.

Graphs of relative departures of average July temperature from their means are shown on Fig. 2. We only consider the past 6,000 yr so that changes in the pollen spectra primarily reflect climatic stresses rather than migrational histories of the different taxa. This statement may not be accurate for Labrador where an unusually late migration of conifers several thousand years after deglaciation took place^{13–15}.

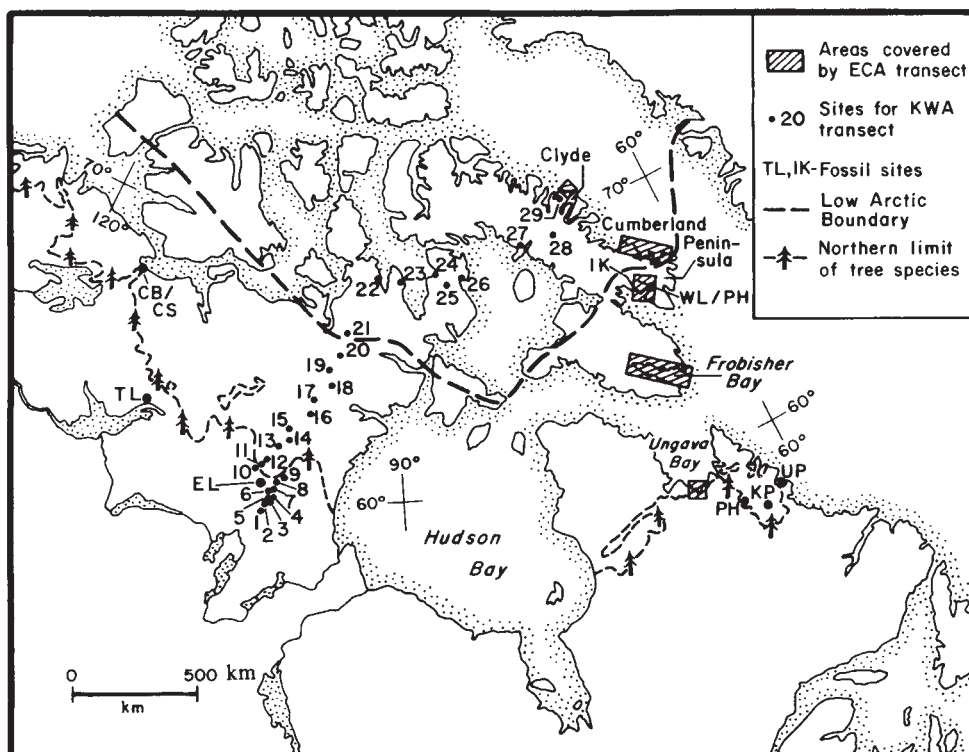
The most important information in the time-series is the negative trend in the relative departures of July temperatures that begins between 3,000 and 2,500 yr BP.

Table 1 Information on the transfer function equations

	ECA-1	MECA-1B	MECA-2	KWA-1	MKWA-1B	MKWA-1
No. of samples	18	18	87	29	29	293
No. of taxa entered	19	19	19	13	13	13
No. of taxa selected in the equation	4	4	4	4	4	4
Multiple r	0.9	0.92	0.76	0.95	0.95	0.89
Standard error ($\pm$)	0.93	0.86	1.3	0.75	0.79	1.06
Names of the critical taxa	NA	<i>Alnus</i> <i>Picea</i> <i>Salix</i>	<i>Alnus</i> <i>Picea</i> <i>Betula</i>	NA	<i>Alnus</i> <i>Picea</i> <i>Betula</i>	<i>Alnus</i> <i>Picea</i> <i>Betula</i>
Reference	6	Gramineae 6	Rosaceae 6	9	Gramineae 9	Gramineae 9

The pollen taxa are in percentages. The climatic parameter estimated is July average temperature (°C). MECA and MKWA designate transfer function equations based on multiple, stepwise regression analysis. The Imbrie–Kipp approach has also been used and gives comparable results^{2,8}; these results are designated ECA and KWA. NA, not applicable.

Fig. 1 Location of the two transects of modern surface pollen samples. Keewatin to Baffin Island sites numbered 1–29; Labrador to Baffin Island sites outlined as rectangles. Treeline and the Low Arctic vegetation boundary shown. Peat sites: EL, Ennadai Lake; TL, Thompson Landing; CB and CS, Coppermine Beach and Saddle; WL/PH, Windy Lake/Pass Head. Lake sediment sites: IK, Iglutalik Lake; UP, Ubluk Pond; KP, Kogaluk Plateau Lake; PH, Pyramid Hills lake.



On Baffin Island, both records show a statistically significant decrease in average July temperature over the period of record (Fig. 2). The local thermal maximum terminated between 5,300 and 5,000 yr ago. The lowest estimated July departure during the past few thousand years occurred between 600 and 200 yr ago, although both records indicate continuously cool summers after ~2,300 yr BP. Present July temperatures are closely predicted by modern surface pollen spectra for this region; hence, the present temperature estimates are as warm, if not warmer, than any values estimated for the past 1,000–2,000 yr. The ages for the Windy Lake peat are based on a uniform sedimentation model. Davis¹¹ argued that a nonlinear relationship better fitted the 14 ¹⁴C dates. Figure 2 shows alternative positions for peaks and troughs of temperature departures. However, the uniform model gives a satisfactory agreement with Iglutalik Lake.

In Labrador¹³, the Ubluk Pond site is our oldest record, and the July temperature departures >6,000 yr BP might be influenced by migrational lags in vegetation. The transfer

functions predict that the major late Holocene cooling did not begin until 2,500 yr ago, and that the local thermal maximum occurred 4,000–3,000 yr BP. The other two sites from Labrador support this interpretation^{13,14}. However, the record from Kogaluk Plateau Lake ends at ~2,500 yr BP, so that late Holocene changes are not recorded at that site.

Four sites have been examined from Keewatin¹². Data from the Coppermine Beach showed no statistically significant trend of departures against age. The Beach site is on a dry, raised beach, whereas the Coppermine Saddle site is wetter.

Most of our sites (Fig. 2) indicated that July temperature departures became, on average, more negative over the last few thousand years. However, this does not preclude rapid climatic changes at certain times. Nichols^{12,16} argued that the pollen evidence from Keewatin favoured a series of synchronous step changes in climate across the mainland of arctic Canada, which were superimposed on a general decline in July temperatures. Certain temperature graphs from our work show evidence for similar rapid changes (Fig. 2). In particular, the very rapid

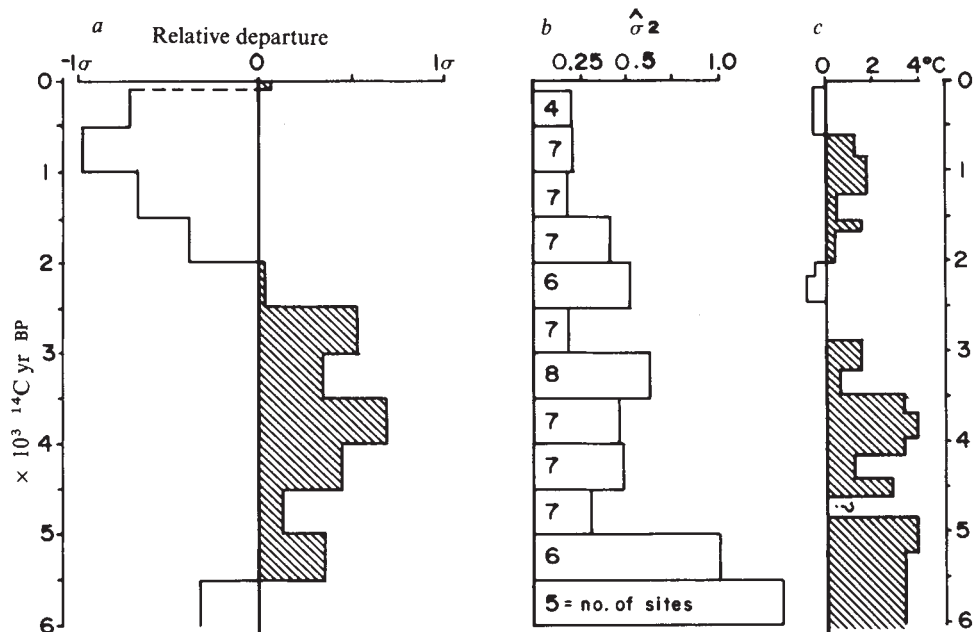


Fig. 2 Time-series of estimated July temperatures at nine sites across northern Canada. Temperatures expressed as relative departures from the smoothed mean of each series. Table 2 lists $\bar{x}$ and s for each series. A–A', and so on, refer to different timings of peaks at Windy Lake depending on which sedimentation model is used.

Table 2 Analysis of temperature data in Fig. 1

Site	Ubluk Pond	Kogalu Pl.	Pyramid L.	Thompson L.	Ennadai L.	Coppermine Beach	Coppermine Saddle	Windy L.	Iglutalik
$\bar{x}$	12.1	13.1	13.2	10.0	9.4	9.25	10.1	6.3	8.0
s	0.7	0.7	0.5	0.8	1.2	0.7	1.2	1.0	0.8
n	22	14	34	59	40	20	23	90	53
Present July temperature ($^{\circ}\text{C}$) ($\pm$)	11 $\pm$	13	13	12.8	12.3	9.3	9.3	7	7

$\bar{x}$ and s are mean and standard deviation for each time series.

cooling that began in the late Holocene is notable. At Coppermine Saddle, a temperature drop of about 2°C in 150 yr between 1,400 and 1,250 yr BP is estimated. At Ennadai Lake, Windy Lake and Ubluk Pond, July temperatures fell $1\text{--}2^{\circ}\text{C}$ close to 2,500 yr BP.

The timing and direction of these changes were determined by averaging the data (Fig. 2) in 500-yr time-blocks and expressing the departures as $\pm$ values about the mean of each series (Fig. 3). If regionally offsetting departures were present, we would expect the average departure to be close to zero. However, regionally synchronous changes in estimated July temperatures can be identified on Figs 2 and 3. Our study area extends across the region covered by one of the main upper atmospheric troughs. As this trough shifts to the east of west, out-of-phase climatic trends may occur, at least on short time scales^{17,18}. However, arguments for and against synchrony of climatic change on the longer time scales of centuries occur^{12,18,19}.

Between 5,500 and 6,000 yr ago, in northern Canada, mean July temperature departures were, on average, negative. July temperature departures then rose to above average values until 2,500 yr BP, with a reversal between 5,000 and 4,500 yr BP (Fig. 3). The highest average departures from July conditions occurred 3,500–4,000 yr BP, which apparently represents a Holocene local thermal optimum for the eastern and central Canadian Arctic. Between 3,000–2,500 and 1,000–500 yr BP, the average departures declined steadily and reached a trough in the latter interval.

Nichols¹² graph of departures of July temperatures from modern values is reproduced on Fig. 3. The major difference between that reconstruction and ours is the interpretation of events after 2,500 yr BP; our reconstruction shows a steady decline to values well below the mean of the nine time-series, whereas Nichols' reconstruction shows less of a decline and, in fact, has temperatures above present between 2,000 and

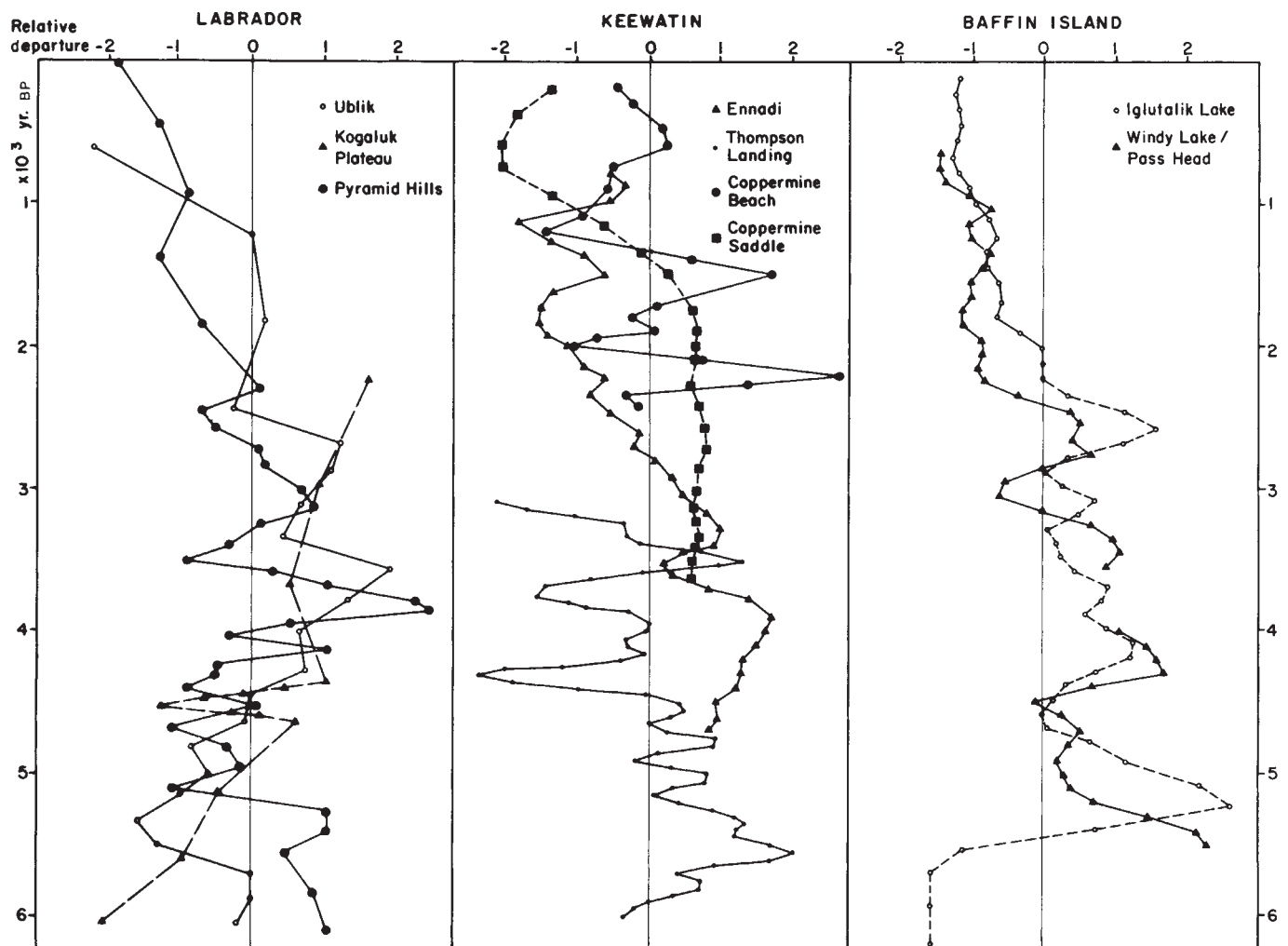


Fig. 3 *a*, Average relative departures for all sites for 500-yr time blocks; shaded area indicates period when relative departures were above the average July temperature ($^{\circ}\text{C}$) for the past 6,000 yr. *b*, Variance of the relative departures, number within each block represents the number of curves available for each interval. *c*, Nichols' model of variations in July temperature ($^{\circ}\text{C}$) for central, northern Canada based on an analysis of fossil pollen spectra. The temperature history of the past 200 yr is inferred and based on comparison of present average July temperatures compared to the fossil record.

600 yr BP. We have yet to examine our data for how far the variance of Fig. 3c is due to pronounced regional differences in our estimated July temperature records, or how far it is associated with uncertainties in the pollen transfer functions, or how much is associated with problems of ^{14}C dating organic poor sediment^{13,20}.

Our data indicate that the most useful northern analogue for a 'warm' summer world during the past 6,000 yr is between 3,500 and 4,000 yr ago. Figures 2 and 3 indicate that July temperatures in the central and eastern Canadian Arctic departed, on average, by about 0.75 standard deviation from the 6,000-yr mean, or $\sim 1^\circ\text{C}$. Locally, estimated July temperature reconstructions are predicted to have been 2°C to 3°C warmer than at present.

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Footprints of a Pleistocene hominid in northern Kenya

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We report here that in 1978, while studying Koobi Fora Formation sedimentary environments and fossils in northern Kenya, we discovered single footprints and trails of large vertebrates in a number of different strata. One of these trails is that of a bipedal hominid, the second known record of early hominid tracks in Africa, the other being that reported at Laetoli, Tanzania¹.

The Koobi Fora Formation is exposed for 2,600 km² along the northeastern shore of Lake Turkana. It is up to 325 m thick and consists of laterally variable facies deposited in lacustrine, lake margin and fluvial environments^{2,3}. Within the Upper Member of the Koobi Fora Formation, in the vicinity of Koobi Fora (Fig. 1a), is a poorly consolidated 10–20-m sedimentary complex of tuffaceous sands and silts designated the Koobi Fora Tuff⁴. This has yielded a scatter of radiometric dates with a preferred estimate of 1.5–1.6 Myr (ref. 5). Correlation based on lithology and stratigraphic position can be made from the Koobi Fora Tuff to the Okote Tuff (Karari area) and Lower/Middle Tuff (Ileret area) to the north^{2,3}, although outcrops between areas are discontinuous (Fig. 1a). The Okote and Lower/Middle Tuffs are also dated at 1.5–1.6 Myr (ref. 5). Biostratigraphic correlations to other dated sequences including

Table 1 Numerical data for the Koobi Fora Tuff hominid tracks

Hominid print	Width (cm)	Length (cm)	Maximum depth (cm)	Orientation	Notes
L1	13.0	26	6.0	80°	Deepest near first toe, morphology poorly preserved
R1	18.0	32	6.0	90°	Deepest under ball of foot, morphology poorly preserved
(L2)	—	—	—	—	Missing, probably due to locally hard substrate
R2	7.0	—	1.5	~87°	Incomplete print, probably heel only
L3	10.5	27	4.0	51°	Includes partial toe prints, deepest at heel
R3	10.0	26	3.0	92°	Probable minor slipping during step
L4	—	—	—	~88°	Partial, superimposed on composite hippopotamus prints
R4	9.5	25	2.5	92°	Greatest depth at heel and along outside edge

R = right foot, L = left foot; numbered from west to east in the direction of movement. Width is measured at the ball of the foot, length from toe to heel along the midline.

the Omo Shungura Formation⁶ and Olduvai Gorge^{7,8} support this date.

There are extensive exposures of the Koobi Fora Tuff in Area 103 (lat $3^\circ 53'\text{N}$, long $36^\circ 15'\text{E}$), 13 km SSE of the Koobi Fora research camp, in a series of fault blocks. In 1978, step trenches revealed over 20 distinct strata with sedimentary structures which, in cross-section, seemed to be imprints of large vertebrate feet on originally soft, muddy substrates⁹. The sediments record marginal lacustrine environments and are vertically complex, with alternating thin units (1–15 cm) of sand and mud. Within the uppermost 1 m, cross-sections of particularly well preserved footprints were found in a unit of sandy mud (Fig. 1b). An area of 1 m² of the upper surface of this unit was cleared of overlying sediment to determine the shapes, in plan view, of the footprints. Excavation revealed several deep, four-toed impressions of hippopotamus feet and a shallow depression resembling a human footprint. Kimolo Mulwa and Bernard Kanunga of the National Museums of Kenya were responsible for the uncovering and recognition of this first hominid track (R4). Excavation of a second 1-m² area exposed two further complete human-like prints in line with the first, confirming the discovery of a hominid trail. Excavation in 1979 expanded the area of exposed bedding surface to 12 m² and revealed two further complete hominid tracks together with those of many other vertebrates (Fig. 1c).

The footprints are in a homogeneous, pinkish-buff sandy mud (sand:silt:clay = 12:59:29) about 12 cm thick, interbedded with ripple-laminated, fine pumaceous sands of comparable thickness, with sharp upper and lower contacts. The underlying muddy sand unit has slight, concave-upward deformation of laminae beneath the hippopotamus footprints, which penetrate through the sandy mud to within 1 cm of its base. This deformation shows the effects of the weight of the animals and indicates that the sand under the sandy mud was relatively compact when trampled. Muddy sand (sand:silt:clay = 81:10:9) infillings of the footprints are slightly coarser than the overlying sand and include fine silt laminae, concentrations of heavy minerals, and in some cases abundant sand-coated mud-clasts. The footprint layer has an irregular upper surface with higher rims around the deeper tracks, apparently a result of displacement of the mud as the foot penetrated the substrate. There seems to have been no overall scouring before burial of the mud; the sand-bearing currents simply filled and buried the tracks. The sandy mud was viscous enough to form thin, 10-cm-high walls between tracks, and it is doubtful that these would have survived even slight erosion by current action. There are no mudcracks in the footprint unit as evidence for subaerial exposure, although a sample of the sandy mud readily developed cracks when wetted and dried. We conclude that the footprints were formed when the mud surface was submerged under quiet

water. The track of a small wading bird indicates that the water was shallow, <10 cm deep.

On the 12 m² of excavated surface, 89 distinct depressions between 2 and 23 cm deep can be identified as the tracks of large vertebrates (Fig. 1c). Of these, 22 indicate a foot similar to that of modern hippopotamus, and in 15 there are clear impressions of toes. In some cases the toe-marks show that the animals slipped while stepping in the mud. Three distinct trails record one hippopotamus going north-east and two going west. The larger prints (25–32 cm wide) can be attributed to the large fossil hippopotamus, *Hippopotamus gorgops*, recognized from the Koobi Fora Formation at that time¹⁰. The smaller ones (18–

20 cm wide) could be due to juveniles of this species or may belong to the fossil pigmy species, *Hippopotamus aethiopicus*¹⁰. Four of the other, smaller tracks resemble those of modern large Bovidae (the size of a topi, *Damaliscus korrigum*, about 120 kg) but are not sufficiently well preserved for definite identification. There are also two distinct bird prints, one large and one small. The larger is a clear, three-toed impression 20 cm wide, similar in size and morphology to that of a modern bustard or crane. The smaller, which is 2.0 cm wide, resembles tracks of a small shore bird such as a plover.

Five of the depressions on the surface are complete tracks made by the feet of a bipedal hominid (Fig. 1c). Two additional

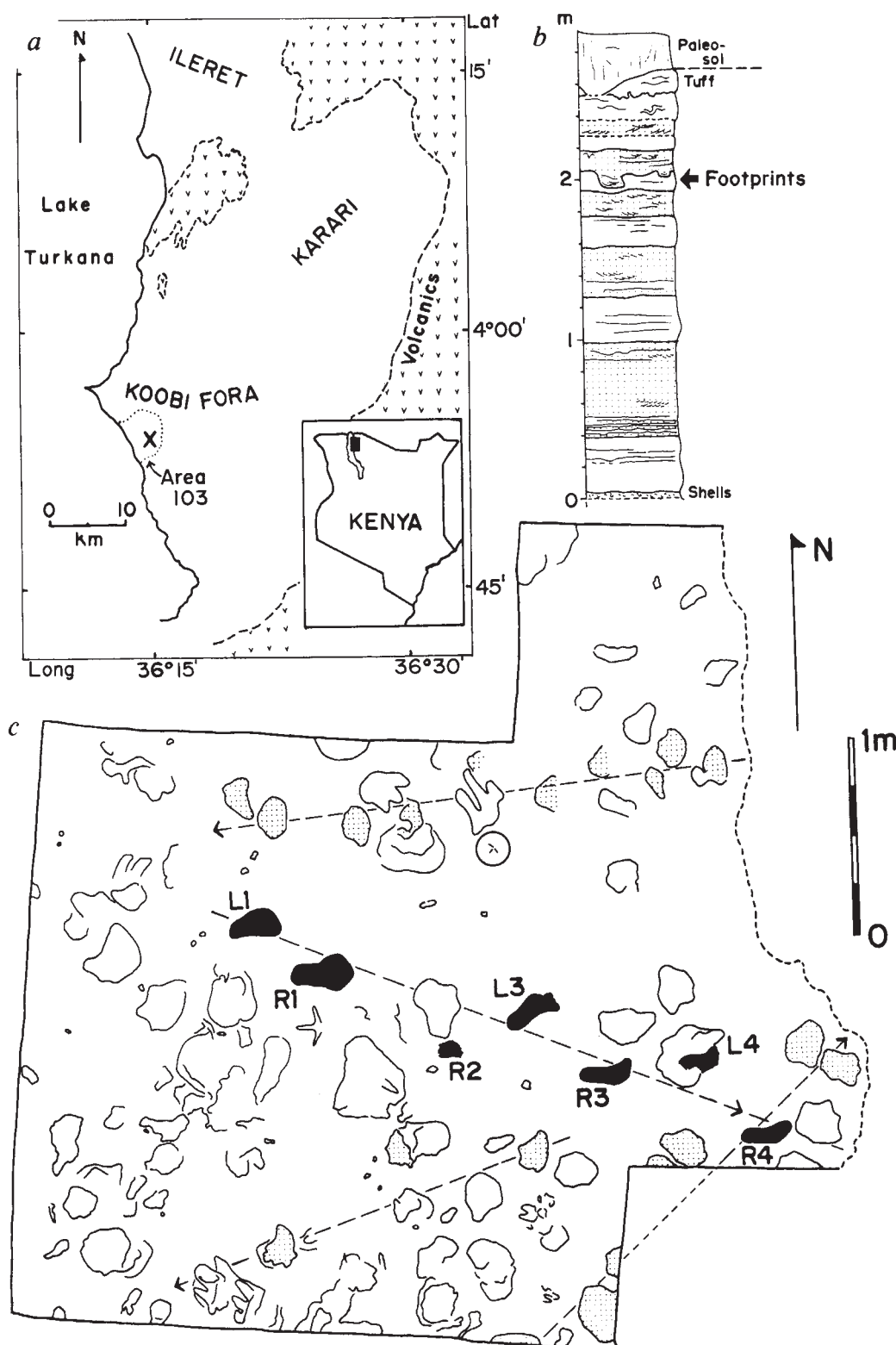


Fig. 1 Map showing location of hominid footprint locality (x) in relation to Lake Turkana and areas of outcrop of the Koobi Fora Tuff and its lateral equivalents in the north. Inset shows location in Kenya. **b**, Stratigraphic section showing upper 2.6 m of the Koobi Fora Tuff and the position of the footprint unit (arrow). Muddy sand units stippled, sandy mud blank. Heavy dashed line indicates top of Koobi Fora Tuff. **c**, Map of the excavated surface of the footprint layer, with hominid footprints (shown in black). Dashed lines indicate direction of movement for the more distinct trails (light stipple in hippopotamus footprints forming the trails). Track of small wading bird is circled.



Fig. 2 Two of the best-preserved footprints in the 1.5-Myr old trail discovered in northern Kenya, framed by the modern feet of *Homo sapiens* (27 cm long). These prints are referred to as L3 and R3 in Fig. 1c. Scale bar, 15 cm. (Photograph by Léo F. Laporte.)

tracks can be attributed to the same individual although they would not be identifiable as hominid if observed separately from the trackway defined by the other five footprints. The complete tracks are shallower towards the east, in the direction of movement (Table 1). The westernmost tracks (L1 and R1) are oversized and poorly shaped compared with the others, and bear evidence of minor erosion and slumping before burial. The absence of an L2 track is puzzling, but may be due to a patch of substrate that was compact enough to bear the weight of the hominid or to local scouring before burial. More hominid tracks would probably be uncovered if the excavation were extended westward along the line of the trackway, although the increasing amount of overburden would make that a difficult operation.

From the dimensions of the best-preserved tracks, L3, R3 and R4, we estimate that the hominid foot was about 26 cm long and 10 cm wide (Table 1). These fall close to average dimensions for American *Homo sapiens* males¹¹. Other tracks in the series are enlarged or incomplete and cannot be used to reconstruct foot shape. The height of the hominid can be estimated at 1.6–1.7 m based on the relationship of foot length to stature for American negro and white males¹¹, and 1.8 m based on those for San bushmen¹². The hominid prints are longer and relatively narrower than those recorded at Laetoli, which average 18.5 cm long and 8.8 cm wide (trail 1) and 21.5 × 10 cm (trail 2)¹.

In the study of footprints, a standard definition of 'stride' is the distance from a point on one foot (left or right) to the same point on the next impression of the same foot¹³. The length of the stride of the hominid is about 80 cm, less than that of modern humans with feet of comparable size during normal walking¹⁴. This, along with the orientation of the feet, seems to indicate a hesitant, somewhat sideways progression across a slippery surface with one mis-step (L4) into a deep hippopotamus track, which may have been concealed by turbid water.

At least two bipedal hominid taxa are represented by fossil bones from the Koobi Fora Formation at the time of deposition of the footprint unit: *Homo erectus* and *Australopithecus robustus*^{15–17}. In Area 103, only the remains of *H. erectus* have been found in the Koobi Fora Tuff although in the Karari and Ileret regions both taxa occur in units correlated with the Koobi Fora Tuff. The *H. erectus* specimens from the Koobi Fora Tuff occur within its lower 6 m; none has been found within the upper part where the hominid trackway is recorded. It is not possible to determine which hominid made the footprints.

Trails of early hominids known from the Pliocene Laetoli Beds in northern Tanzania were preserved in subaerial volcanic ash strata dated at 3.60–3.75 Myr (refs 1, 12). They occur with a large series of tracks and trails representing a wide diversity of savannah animals. The hominid tracks are thought to belong to the same species as hominid fossils from the Laetoli Beds,

designated as *Australopithecus afarensis*^{1,12,18}. In contrast, the Koobi Fora footprints occur on a muddy lake margin with tracks of a limited number of semi-aquatic vertebrates. The Koobi Fora footprints contribute a reference point on hominid foot morphology, locomotion, behaviour and ecology 2 Myr younger than the Laetoli occurrences and ~1 Myr older than late Pleistocene human footprints in Europe¹⁹.

A latex mould and plaster cast of the Koobi Fora hominid footprints described here is stored at the National Museums of Kenya, Nairobi. We thank the Government of Kenya, the National Museums of Kenya and the International Louis Leakey Memorial Institute for African Prehistory for their cooperation and support. Richard Leakey, Meave Leakey, Glynn Isaac, Kamoya Kimeu and Tim White are acknowledged for their suggestions and help. The excavation crew, headed by Muteti Nume and Mukilya Mun'goka, were directly responsible for the technical work that led to the discovery of the trackways. Hilde Schwartz and Mahmood Raza assisted with the excavation work. Palaeoecological research was sponsored by NSF grant EAR77-23149 and is a part of the overall Koobi Fora Research Project, which is supported by grants from the National Geographic Society, the NSF and the W. H. Donner Foundation.

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Relationship between fungus and alga in the lichen *Cladonia cristatella* Tuck

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The nature of the lichen symbiosis is not clear. It is generally thought to be mutualistic but this concept is not supported by experimental evidence¹. Early workers^{2,3} considered that lichens represented algae parasitized by fungi—as evidence, they noted algal cells in a lichen thallus that were dead or penetrated by fungal haustoria. Others, however, cited the seemingly healthy and long-lasting nature of lichens as evidence of mutualism. As we report here, our observations of artificial syntheses of the mycobiont *Cladonia cristatella* ('British soldiers') with different algae suggest that the relationship in this lichen is one of controlled parasitism. The mycobiont formed squamules mostly with algae related to its natural phycobiont, an indication perhaps of a long period of co-evolution between the symbionts of this lichen.

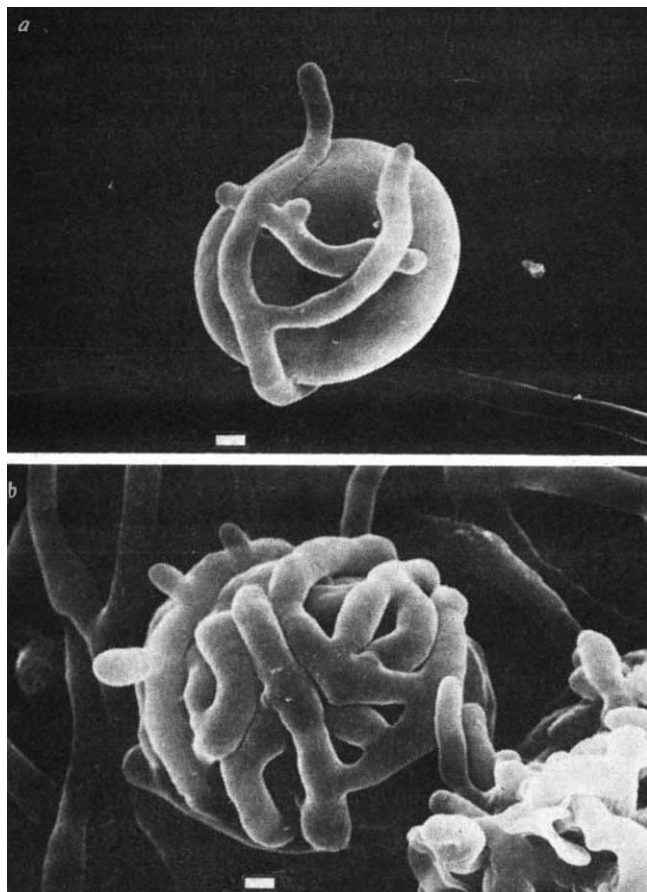


Fig. 1 Envelopment of cells of *Trebouxia erici* by mycobiont. With the aid of a dissecting microscope, mica strips with colonies of resynthesized lichens were cut into 13-mm pieces and placed in Petri dishes filled with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, and fixed for 30 min. The colonies were rinsed briefly in phosphate buffer and then fixed for 30 min at room temperature in 1% OsO₄ in 0.1 M phosphate buffer. The specimens were rapidly dehydrated through an ascending alcohol series and dried by the critical point method. Specimens were cemented to specimen stubs, sputter coated with approximately 100 Å of gold, and examined at 20 KV in a JEOL JSM 35U scanning electron microscope. *a*, Algal cell encircled by branches from one hyphal filament. A thin gelatinous matrix around the alga binds the hyphae. *b*, Algal cell almost fully enveloped by hyphae. Haustorium has penetrated the cell at the top and along one hypha are three new branches. Scale bars, 1 µm.

Procedures used are described in Table 1 legend. After 2–3 weeks of axenic culture, the fungus formed squamules with five species of *Trebouxia* phycobionts and presquamules with *Trebouxia italiana* and *Friedmannia israeliensis*. The phycobionts were isolated from 13 different lichens. The fungus did not form lichenized structures with 17 algal symbionts, the free-living algae *Chlorella vulgaris* and *Pleurastrum terrestre* or unlichenized representatives of the Chlorococcales (Table 1). The fungus was not specific in its attachment, encircling glass beads (10–15 µm in diameter) in the same manner as algal cells, although the hyphae could be easily dislodged from the beads.

Algal cells were covered by a substance which bound the fungal hyphae (Fig. 1a) similar to that seen between the symbionts of the natural lichen and in a different lichen synthesized previously⁴. When initial contacts were established, the fungus, depending on the alga contacted, formed lichenized associations or destroyed the cells with its haustoria. Initial contacts (Fig. 1a) were followed by further encirclement of the algae, some cells being almost completely enveloped (Fig. 1b). After the fungus had secured small groups of algal cells and formed presquamules (Fig. 3a), a network of hyphae formed over the presquamules (Fig. 3b) linking them into a common structure. Thickened and fused hyphae with irregular extra-

hyphal material developed first in small patches and later over a broader area of the mixed culture, forming the upper cortex and network of hyphae which covered the squamules. Further differentiation resulted in an algal layer and medulla. The morphology and dimensions of the thallus layers were identical to those of the natural squamules.

Table 1 Interactions between the mycobiont *Cladonia cristatella* (s.s.31) and various algae

Algae lichenized by mycobiont	
<i>Friedmannia israeliensis</i> * (UTEX 1181; non-lichenized, presquamules only)	<i>Trebouxia glomerata</i> (<i>Cladonia boryi</i>)
<i>Trebouxia erici</i> (UTEX 910; <i>Cladonia cristatella</i>)	<i>Huillia albocaerulescens</i> UTEX 894; <i>Stereocaulon saxatile</i>)
<i>Trebouxia excentrica</i> (<i>Cladonia bacillaris</i>)	<i>Trebouxia italiana</i> (CCAP 219/5b; <i>Xanthoria parietina</i> , presquamules only)
<i>Cladonia leporina</i>	<i>Trebouxia magna</i> (UTEX 902; <i>Pilophoron acicularis</i>)
<i>Cladonia subtenuis</i>	<i>Trebouxia pyriformis</i> (<i>Stereocaulon pileatum</i>)
<i>Lecidea metzleri</i>	
<i>Lepraria</i> sp.	
UTEX 1714; <i>Stereocaulon dactylophyllum</i> var. <i>occidentale</i>)	
Algae parasitized by mycobiont	
<i>Bracteacoccus minor</i> * (UTEX 66; non-lichenized)	<i>Pseudotrebouxia jamesii</i> (<i>Schaereria tenebrosa</i>)
<i>Chlorella vulgaris</i> (non-lichenized)	<i>Pseudotrebouxia potteri</i> (<i>Pertusaria</i> sp.)
<i>Chlorococcum ellipsoideum</i> * (UTEX 972; non-lichenized)	UTEX 900; <i>Rhizoplaca chrysoleuca</i>)
<i>Chlorococcum gelatinosum</i> * (UTEX 1773; non-lichenized)	<i>Pseudotrebouxia showmanii</i> (<i>Lecanora hageni</i>)
<i>Myrmecia biatorellae</i> (<i>Dermatocarpon tuckermanii</i>)	<i>Pseudotrebouxia usneae</i> (<i>Usnea filipendula</i>)
<i>Nautococcus pyriformis</i> * (UTEX 125; non-lichenized)	<i>Pseudotrebouxia</i> sp. (<i>Aspicilia calcarea</i>)
<i>Nautococcus terrestris</i> * (UTEX 1794; non-lichenized)	<i>Astrolapa</i> sp.
<i>Neochloris terrestris</i> * (UTEX 947; non-lichenized)	<i>Caloplaca holocarpa</i>
<i>Nostoc punctiforme</i> (<i>Peltigera canina</i>)	<i>Diploschistes scruposus</i>
<i>Pleurastrum terrestre</i> (UTEX 333; non-lichenized)	<i>Lecania</i> sp.
<i>Pseudochlorella</i> sp. (<i>Lecidea granulosa</i>)	<i>Lecanora tenera</i>
<i>Lecidea scalaris</i> (<i>Stereocaulon strictum</i>)	<i>Lecidea crystallifera</i>
<i>Pseudotrebouxia aggregata</i> (<i>Lecidea fuscoatra</i> UTEX 180; <i>Xanthoria</i> sp.)	<i>Lecidea sarcogonoides</i>
<i>Pseudotrebouxia corticola</i> (UTEX 909; non-lichenized)	<i>Omphalodium arizonicum</i>
<i>Pseudotrebouxia decolorans</i> (UTEX 781; <i>Buellia punctata</i>)	<i>Parmelia tinctorum</i>
<i>Pseudotrebouxia galapagensis</i> (<i>Ramalina</i> sp.)	<i>Rhizocarpon geographicum</i>
<i>Pseudotrebouxia gigantea</i> (<i>Caloplaca cerina</i>)	<i>Toninia caeruleonigricans</i>
<i>Pseudotrebouxia higginsiae</i> (<i>Buellia straminea</i>)	<i>Xanthoria parietina</i>)
<i>Pseudotrebouxia impressa</i> (UTEX 892; <i>Physcia stellaris</i>)	<i>Radiosphaera dissecta</i> * (UTEX 1187; non-lichenized)
	<i>Spongiocloris excentrica</i> * (UTEX 108; non-lichenized)
	<i>Spongiocloris llanoensis</i> * (UTEX 1245; non-lichenized)
	<i>Trebouxia antipata</i> (UTEX 903; <i>Parmelia rudecta</i>)
	<i>Trebouxia crenulata</i> (CCAP 219/lb; <i>Parmelia acetabulum</i>)
	CCAP 219/2; <i>Xanthoria aureola</i>)
	<i>Trebouxia gelatinosa</i> (UTEX 905; <i>Parmelia caperata</i>)

Algal and fungal symbionts were separated into culture using standard isolation techniques. The algal symbionts were grown on Bold's basal medium (BBM)x3N + 1% glucose agar slants. Single spore isolates of the fungal symbiont were cultured in Lilly and Barnett liquid medium or malt-yeast extract broth liquid medium¹³. The fungal cultures were centrifuged at 10,000 r.p.m., washed with distilled water and re-centrifuged at the same speed. Clumps of hyphae were taken from the pellet and mixed with different algae. These symbiont mixtures were inoculated onto the surface of newly cleaved mica strips (1×7.5 cm) which had been soaked in BBMx3N solution. Each strip was located in a 125-ml Erlenmeyer flask; one end of the strip was embedded in 20 ml of plain 2% agar and the other end rested against the flask. The flask was plugged with non-absorbent cotton covered with aluminium foil. The relative humidity in the flask was about 95%. Incubation of the flasks was at 3000 lx, 12/12 light-dark cycle, at 21 °C during the light period and 19 °C in the dark period. Scanning electron microscope observations were made 2 months after inoculation.

* Mycobiont used in these crosses was isolate no. 6.

Haustoria were common in the natural and synthetic lichens (Fig. 2a), but in the former, about twice the proportion of algal cells was penetrated than in the latter (Table 2). Algae in the newly synthesized squamules were probably dividing more rapidly than those in the natural thalli, thus being less susceptible to fungal contacts.

Algae that were incompatible with the fungus (Table 1) were parasitized and destroyed (Fig. 2b). These included many green and one blue-green phycobionts and non-lichenized forms. Because initial binding of the fungal hyphae to algal cells seems to be similar in all cases, compatibility must be a secondary phenomenon. Perhaps there is algal resistance to fungal parasitism; that is, some algae may succumb more slowly than others to particular fungi.

In *C. cristatella*, the relationship between fungus and alga seems to be controlled parasitism. In the natural lichen, although more than 50% of the phycobiont cells were penetrated by haustoria, the algal population seemed healthy. In our successful laboratory syntheses, we saw algal cells apparently killed by haustoria and hyphae growing through them. Therefore, any resistance that compatible algae may have to the fungus is not permanent. In nature, a balanced relationship may have evolved between the symbionts. It is possible that the parasitism of the natural phycobiont is balanced by proliferation of the algal cells. Others have suggested controlled parasitism⁵⁻⁷ and it may be a general feature of lichens.

The role of the haustoria is not clear. Evidence indicates that they do not transfer metabolites from alga to fungus^{8,9}. They might be vestigial structures of ancestral fungi that were more actively pathogenic on other plants. A relationship between lichen fungi and unlichenized fungal parasites is suggested by the concentric bodies, cellular organelles of unknown function

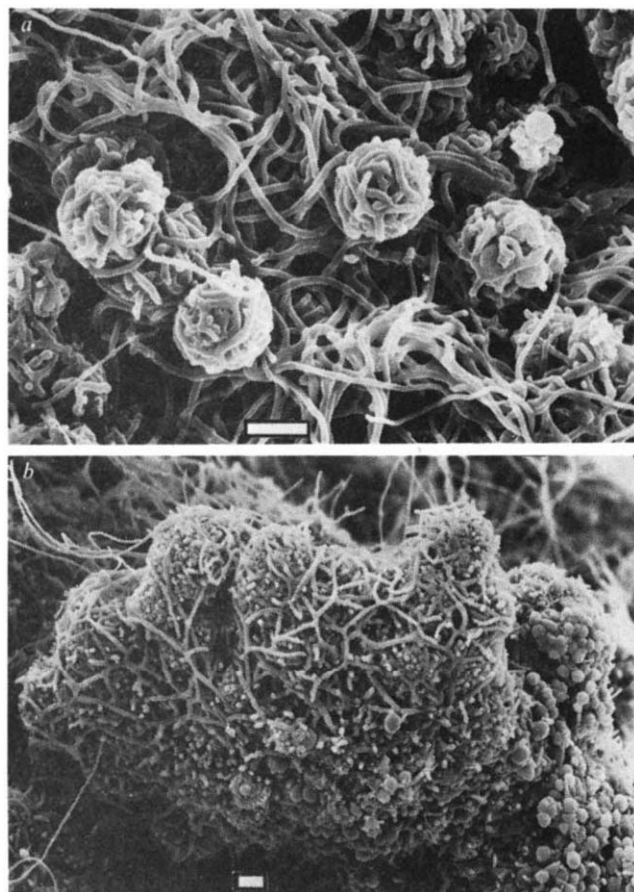


Fig. 3 Development of lichen squamules. See Fig. 1 legend for procedures. *a*, Presquamules formed by algal cells enveloped by mycobiont. *b*, Presquamules linked together by a network of superficial hyphae. Scale bars, 10 µm.

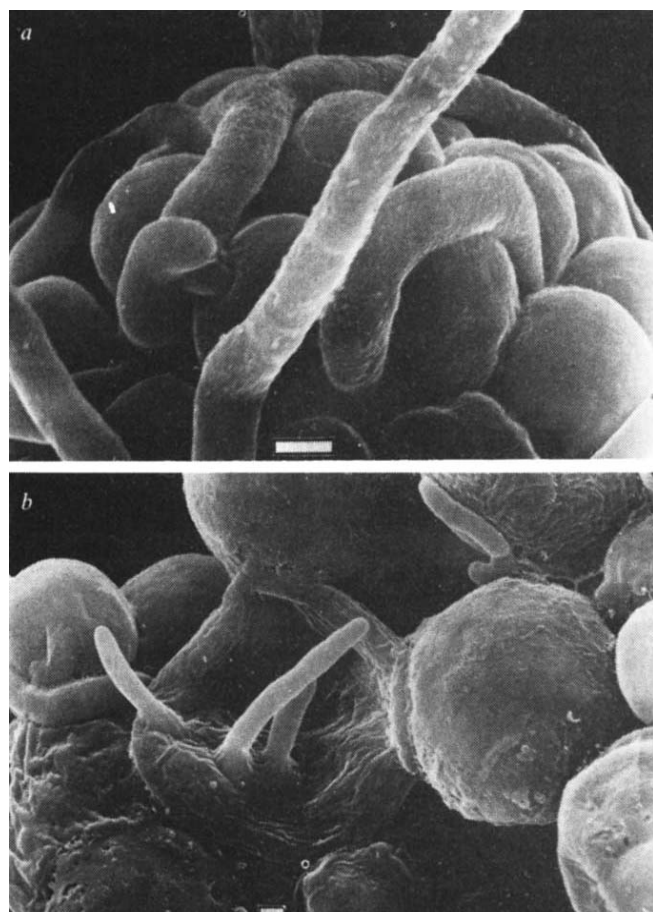


Fig. 2 Fungal intrusions into algal cells. Procedures used were as described in Fig. 1 legend. *a*, Cell of *Trebouxia erici* showing invaginated wall around haustorium. *b*, Three hyphae growing out of collapsed cell of *Trebouxia gelatinosa*. Scale bars, 1 µm.

found in numerous lichen fungi and ascomycete plant pathogens.

Some algal cells in the early stages of the successful syntheses were almost completely covered by fungal hyphae, which may be a response to the nutritional needs of the fungus. The mica substrate contained only mineral supplements; thus, the algal cells provided the only source of organic nutrients for the fungus. Farrar¹⁰ estimated that 90% of the carbon fixed in photosynthesis by natural lichens is released by the algae but this release virtually ceases when the algae are removed from the

Table 2 Frequency of haustoria in natural and resynthesized squamules of *Cladonia cristatella*

	Total cells observed	Cells penetrated by haustoria	% Cells penetrated
Natural lichen			
Squamule 1	200	113	57
Squamule 2	100	65	65
Squamule 3	184	107	58
Resynthesized lichens			
<i>C. cristatella</i> s.s.8 × <i>Trebouxia excentrica</i> (<i>Cladonia leporina</i>)	200	54	27
<i>C. cristatella</i> s.s.8 × <i>Trebouxia erici</i>	100	24	24

Squamules were placed in a drop of Loeffler's methylene blue or lactophenol cotton blue and gently crushed with a cover slip using sufficient pressure to rupture vegetative cells. The ruptured cells extruded their contents and left only the empty shell. Haustoria adhered strongly to the algal wall and were not dislodged by the crushing. Haustoria were readily visible because there were no algal contents to mask their presence and they stained whereas the algal wall remained unstained. Empty algal cells over 5 µm were counted randomly during transects from one side of the slide to the other. Crushed cells were not counted.

symbiosis. The algae we used were growing separately in culture and presumably released little or no carbohydrate. The initial extensive contacts of algal cells by fungal hyphae may be necessary to re-establish permeability of the algal cells. We did not find an extensive hyphal covering of algal cells in the natural lichen. Collins and Farrar⁹ reported that in *Xanthoria parietina* only 20% of the algal wall was in contact with fungal walls. This minimal fungal coverage may be related to the large release of organic material by the alga in the symbiotic condition.

The lichenization of the free-living alga *F. israeliensis* proceeded only to the presquamule stage. The mixed culture showed numerous patches of dead algal cells killed by fungal intrusions. Cells of *P. terrestre* were enveloped by hyphae but presquamules did not form because the algae could not survive association with the fungus. *Trebouxia* belongs to the order Chlorococcales. *Friedmannia*, like *Pseudotrebouxia*, is in the Chlorosarcinales because it has vegetative cell division and *Pleurastrum* is in the Chaetophorales. Although they occupy divergent taxonomic positions, these algae have similar zoospores and division stages of zoosporogenesis^{11,12}.

Our scanning electron microscope observations of the natural lichen showed many bacteria on the surface of the squamules. However, the axenic development of squamules in our cultures revealed that bacteria were not involved in the morphogenetic development of this lichen.

Syntheses took place in constant humidity (about 95%) in a closed flask. Other than the initial drying after the wet fungus

was placed onto the mica strip, the culture did not dry further; thus, the lichen developed despite the absence of drying and wetting cycles thought to be essential in lichen synthesis.

Most chemical substances of natural lichens are products of the symbiosis. Analyses (TLC and ion-HPLC) of our synthetic lichens revealed the presence of natural secondary products. *C. cristatella* s.s. $\times$ *Trebouxia erici* showed barbatric acid, 4-*O*-demethylbarbatric acid, obtusatic acid, norobtusatic acid, 3- α -hydroxybarbatric acid, didymic acid and rhodocladonic acid. *C. cristatella* s.s. $\times$ *F. israeliensis* showed barbatric acid, obtusatic acid and 4-*O*-demethylbarbatric acid.

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Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat

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All reef-forming, or hermatypic, corals harbour photosynthetic endosymbiotic algae called zooxanthellae^{1–5}, which are assumed to be predominantly a single dinoflagellate species, *Gymnodinium microadriaticum* Freudenthal⁶. The zooxanthellae are essential for the well-being of their hosts^{7–9}; nevertheless, little is known about how light affects the symbiotic association, especially regarding the numbers of zooxanthellae, their photosynthetic responses, and their overall productivity^{10–14}. On the reefs of the Gulf of Eilat, *Stylophora pistillata* is an abundant hermatypic coral¹⁵; it is unique in that region in that it can adapt to a wide range of light intensities. In the high light intensities of lagoons or the upper areas of reefs, the corals are markedly lighter in colour than those living under ledges, in grottos, or near the reef floor (~15 m; Fig. 1). We report here on the biochemical and physiological adaptations of *S. pistillata* to variations in light intensity spanning more than two orders of magnitude.

Specimens of *S. pistillata*, adapted to light and shade regimes, were collected from a remote reef near Nabq in the Sinai Peninsula (28.20° N, 034.95° E) and taken immediately to a lagoon near a shore-based laboratory. The corals were kept in shaded (~10% incident light), submerged baskets in the lagoon until used. Within 2 h of collection, samples were weighed and photographed against a calibrated grid, and chlorophylls were extracted by freezing in liquid N₂ and thawing in 90% acetone on a reciprocating shaker under dim light for 1 h. Extraction was

considered complete when little or no chlorophyll fluorescence was observed on the coral branches under long wavelength UV light¹⁶. The absorption spectra of the acetone extracts were scanned from 400 to 750 nm on a Beckman DB-G grating spectrophotometer and chlorophylls *a* and *c* were calculated according to equations of Jeffrey and Humphrey¹⁷. Soft tissue protein, extracted in boiling NH₃OH, was measured by the method of Lowry¹⁸ using bovine serum albumin as a standard. To determine coral surface areas, photographs were made of individual branches (of known weight) against a calibrated grid and projected onto graph paper. The two-dimensional surface areas were obtained by tracing the coral branches and weighing the paper cutouts of the branches. These two-dimensional areas (that is, cross-sectional areas) were multiplied by π to estimate three-dimensional surface areas, assuming that the coral branches approximate a series of cylinders.

Zooxanthellae were removed from branches with a Water Pik (Teledyne)¹⁹, suspended in fresh seawater, and counted by light

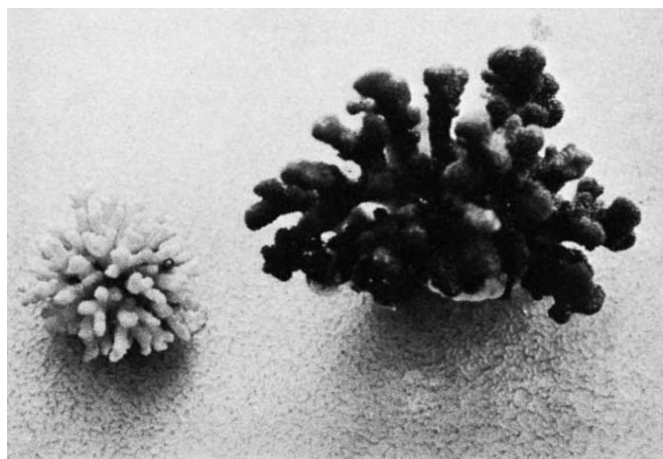


Fig. 1 Photograph of light-adapted (left) and shade-adapted specimens of *Stylophora pistillata* collected from the coral reefs in the Gulf of Eilat.

Table 1 Characteristics of pigment and protein composition of light-adapted, shade-adapted and transplanted individuals of *S. pistillata* and zooxanthellae

Coral type	*% I_0	n	Whole-coral characteristics				Zooxanthellae characteristics			
			†g cm ⁻²	Protein (mg cm ⁻²)	Chl a (µg cm ⁻²)	Cell density (cells cm ⁻² × 10 ⁶)	Chl a (pg per cell)	Chl a/P ₇₀₀ (Molar ratio)	P ₇₀₀ content (pmol per cell × 10 ⁻⁶)	
Shade-adapted	0.3–1.0	10	2.2 ± 0.3	1.9 ± 0.3	14.2 ± 4.0	1.7 ± 0.3	8.3 ± 0.5	1,650 ± 175	5.7 ± 0.8	
Light-adapted	50–90	11	4.1 ± 0.6	1.8 ± 0.6	3.6 ± 1.1	1.6 ± 0.3	2.2 ± 0.3	425 ± 50	5.9 ± 0.5	
Ratio (shade/light)	~200		1.9	1.0	3.9	1.1	3.7	3.9	1.0	
Light-to-shade transplant	From 50–90 To 0.3–1.0	3	4.1 ± 0.6	1.9 ± 0.6	11.2 ± 5.1	1.6 ± 0.2	6.9 ± 1.2	1,250 ± 350	6.2 ± 1.0	
Ratio (L → S transplant/light)	~200		1.0	1.0	3.1	1.0	3.1	2.9	1.1	
Shade-to-light transplant	From 0.3–1.0 To 50–90	3	2.2 ± 0.3	1.9 ± 0.5	3.2 ± 0.9	1.4 ± 0.3	2.3 ± 0.5	450 ± 75	5.7 ± 0.6	
Ratio (S → L transplant/light)	~1		1.9	1.0	0.9	0.9	1.0	1.1	1.0	

The values for transplanted corals are given after 30 days of adaptation to the new light regime. (Values reported are means ± s.e. where applicable.)

* Range of the per cent of the surface light intensity.

† Surface area to mass ratio.

microscopy with a haemocytometer. Suspensions were filtered onto Whatman GF/C glass fibre filters; chlorophylls were extracted by grinding the filters in 90% acetone and quantified spectrophotometrically.

To determine photosynthetic responses, coral branches were placed in 250-ml Wheaton bottles in a constant temperature bath with a self-stirring oxygen electrode (Yellow Springs Instruments, model 5420A) connected to a 356H operational amplifier. Light was provided by a 500 W quartz halogen source and attenuated with Wratten neutral density filters. To provide more even illumination, a mirror was placed directly behind the branches. The size and number of photosynthetic units (PSUs) were estimated by measuring chlorophyll *a*/P₇₀₀ ratios with an Aminco DW-2a spectrophotometer^{20,21}. Light intensities were measured *in situ* with a Lambda LI 185 quantum meter interfaced with a 192S underwater sensor.

S. pistillata can be divided into two major morphological groups: light- and shade-adapted individuals (Table 1). The former have thinner and more numerous branches (Fig. 1), giving a ratio of surface area/weight 1.9 times greater than that of shade-adapted corals. In most cases shade-adapted colonies are subspherical, and only rarely have we observed the flattening previously described^{22,23}. Protein/surface area ratios were remarkably constant for all individuals, however, and it is inferred that ratios of protein per gramme are directly proportional to surface areas per gramme for a given coral species.

Shade-adapted corals contained 7.4 times more chlorophyll *a* per gramme (3.9 times more per square centimetre) than those adapted to high light intensities. Differences in pigmentation reflect the intracellular pigment content of the zooxanthellae and cannot be attributed to differences in numbers of zooxanthellae per surface area of coral (Table 1). Those differences were primarily associated with changes in the number of chlorophyll *a* molecules per photosystem I reaction centre, P₇₀₀. The number of reaction centres was virtually constant in light- and shade-adapted zooxanthellae, suggesting that adaptation is mediated by changes in the size, rather than the number of PSUs, as has been observed in free-living unicellular algae^{24,25}. The range of sizes of PSUs observed in *G. microadriaticum* (approximately 400–1,800 chlorophyll per P₇₀₀) is similar to those found in free-living dinoflagellates^{24,25}, but is larger than for chlorophytes (approximately 450 chlorophyll per P₇₀₀)²⁵ and higher plants (approximately 350–500 chlorophyll per P₇₀₀)²⁶. Such changes were associated with changes in the photosynthetic responses to changes in irradiance (that is, P–I curves, Fig. 2). On the basis of coral surface area, light-adapted specimens had lower photosynthetic capacities and higher compensation light intensities than shade-adapted individuals (Fig. 2a). Instantaneous light-saturated net photosynthetic O₂ production by zooxanthellae exceeded coral respiratory demands in both light- and shade-adapted specimens. In neither was photo-

inhibition observed, even at intensities approaching 2,100 µeinsteins m⁻² s⁻¹ (full noon sunlight in the summer in the surface waters of the Gulf of Eilat). When P–I curves are calculated on the basis of chlorophyll content (Fig. 2b), maximum photosynthetic capacities were not significantly different in light- or shade-adapted corals. However, shade-adapted corals appeared to utilize light more efficiently, as indicated by steeper initial

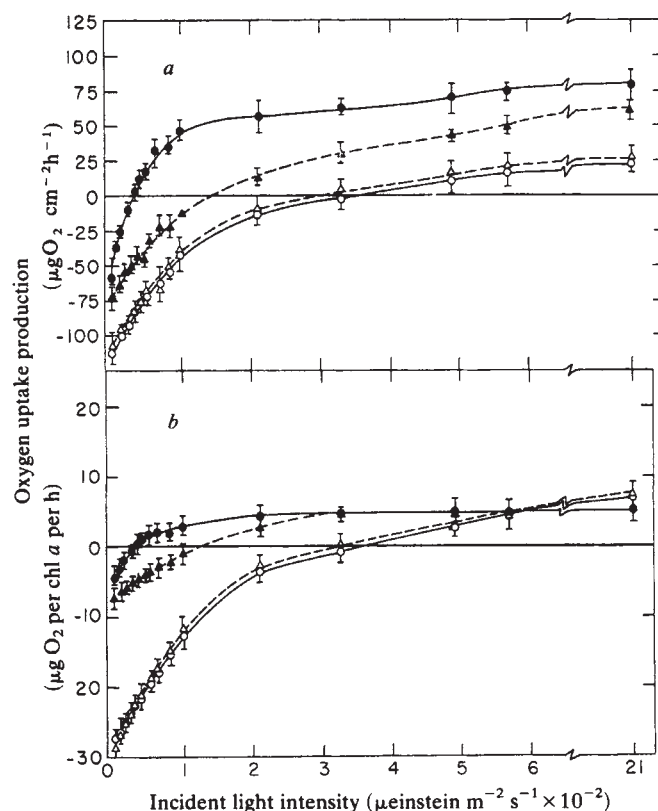


Fig. 2 Photosynthesis-irradiance profiles for light-adapted, shade-adapted, and transplanted *S. pistillata*. *a*, Light curves expressed on a surface area basis: ○, light-adapted; ●, shade-adapted; ▲, light-to-dark transplant after adaptation for 30 days; △, dark-to-light transplant after adaptation for 30 days. *b*, Light curves expressed on a chlorophyll *a* basis. The symbols used are the same as in *a*. Data points represent means and standard deviations ($n = 3$ for light adapted, $n = 3$ for shade adapted, $n = 2$ for dark-to-light transplants, $n = 3$ for light-to-dark transplants). All measurements were made at $28 \pm 1^\circ\text{C}$.

slopes of the P-I curves. These P-I responses were qualitatively similar to those observed in the foliaceous coral *Pavona praelorta* Dana from Enewetak Atoll¹² and are consistent with theoretical arguments that changes in the size of PSUs ought to affect the efficiency of light utilization^{21,24,25}.

Corals were able to adapt when transplanted from one light regime to another (Table 1, Fig. 2). The process was quicker for transplantation from lower to higher light intensities, being effectively complete after 4 weeks. Corals transplanted from high to low light intensities were not fully shade adapted after 4 weeks, but continued to adapt slowly for 6 to 8 weeks. During that period, light-to-shade transplant pigmentation increased in those corals and P-I responses were intermediate between the two extremes. The difference in adaptation time between the two types of transplants may be related to temperature in the two environments^{16,27}.

Previous attempts to transplant corals in this way have not generally been successful as the corals often show signs of physiological stress. This has been interpreted as indicating that light- and shade-adapted corals represent locally adapted populations or ecotypes²³. Usually a particular species appears to be associated with an optimum light regime, and changes in light intensity result in changes in species composition²⁸. Our reciprocal transplant results strongly suggest, however, that changes in coral zonation associated with a gradient in light intensity are not necessarily caused by the inability of zooxanthellae to adapt to wide variations in light regimes. It would seem that in the case of *S. pistillata* the wide range of light intensities tolerated by this coral is not due to zooxanthellae populations of distinct ecotypes as described for the West Indian coral *Montastrea annularis*²³. In particular, the ability of *S. pistillata* to adapt to various light regimes suggests that light- and shade-adapted corals are not always ecotypes and that the niche width of a coral species with respect to light *per se* is not limited by its zooxanthellae.

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Heterozygous advantage expressed through sexual selection in a polymorphic African butterfly

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A field study of the polymorphic African butterfly *Danaus chrysippus* L. at Dar es Salaam, Tanzania (1972-73) indicated that mating may be non-random between forms *dorippus* and *aegyptius*¹ and that seasonal sexual selection occurs². Subsequently, a long-term breeding programme revealed a heterozygous phenotype³ which closely resembles *dorippus*, and allowed estimates of heterozygote frequency in the field⁴. Analysis of mating records (1974-75) shows that males visibly heterozygous at the controlling C locus (from *transiens*)^{3,4} possessed a highly significant mating advantage over both homozygous phenotypes throughout the study period, although pair composition among the three genotypes was random. I propose here that balancing selection, mediated mainly through male competition for mates, makes a decisive contribution to the control of the polymorphism.

Sampling was carried out several times a week for 17 consecutive months (April 1974-August 1975). All captures were marked before release to avoid double counting. The data (Table 1) consist of 340 observed pairings and 1,519 unmated males. As monthly samples were too small to be of much value, they were amalgamated into two annual seasons, one being verdant and relatively cloudy, wet and cool (April-August), when the frequency of *aegyptius* rises rapidly, and the other hot and dry punctuated by a short rainy season (September-March), when *dorippus* predominates⁵. Heterogeneity between mated and unmated samples was tested in $2 \times 3 \times 3$ contingency tables and χ^2 analysed⁶ (Table 2).

The analysis shows that *transiens* males had consistently and very significantly higher success in mating than both homozygous phenotypes and that the effect was independent of season and changes in morph-ratio. Residual heterogeneity was not significant. As CC and Cc butterflies are not always recognizable phenotypically, genotype numbers (Table 3) are estimated by applying a correction for expressivity based on breeding data. The expressivity of c in known heterozygotes is modified by a closely linked B locus: 75% ($n = 173$) of B-Cc and 45% ($n = 129$) of bbCc butterflies were *transiens*⁴, and these values were used to estimate Cc numbers from the number of *transiens* observed. Fitness (Table 3) of CC males was low (9-18%) in both wet seasons, rising to 79% in the dry season. In contrast, cc males had a fitness of 60-64% in the wet seasons, falling to 15% in the dry season. Differential and seasonally alternating mating fitnesses of this magnitude must make a major contribution to the synchronized cycling in morph frequency observed in four successive years (1972-75)^{4,5}. Within seasons, there is no evidence for non-random mating among the three C-locus genotypes (within the mating samples) or for sexual selection in females.

How could such powerful selection for heterozygous males operate? Female preference for them could contribute but is probably not the key factor because it would imply synchronized seasonal switches of the secondary preference (for either CC or cc males) by all three female genotypes—if this were not the case, non-random mating would be detectable in large samples. Cc males (but not females) are significantly larger than both

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Table 1 Numbers of C-locus phenotypes of male *Danaus chrysippus* caught mating and unmated at Dar es Salaam in 1974–75

Period	Condition	Phenotypes			Total	χ^2
		<i>dorippus</i>	<i>transiens</i>	<i>aegyptius</i>		
April–August 1974	Mated	36	52	23	111	11.297
	Unmated	269	187	155	611	$P = 0.0035$
	Total	305	239	178	722	
September 1974–March 1975	Mated	88	47	2	137	13.451
	Unmated	292	113	47	452	$P = 0.0012$
	Total	380	160	49	589	
April–August 1975	Mated	26	40	26	92	12.683
	Unmated	194	117	145	456	$P = 0.0018$
	Total	220	157	171	548	
Overall	Mated	150	139	51	340	26.519
	Unmated	755	417	347	1,519	$P = 1.74 \times 10^{-6}$
	Total	905	556	398	1,859	

homozygotes⁴, which could give them a competitive edge through faster flight. However, the mean forewing length of mated males ($\bar{x} = 37.9$ mm, $n = 161$) did not differ significantly from those unmated ($\bar{x} = 38.0$ mm, $n = 881$) between October 1974 and September 1975; thus selection based on size alone is ruled out. Alternatively, the male aphrodisiac pheromone, essential in courtship^{7–9}, might differ qualitatively or quantitatively between genotypes, although this possibility received no support from a chemical analysis of 50 pairs of hairpencils and wing pouches from each genotype (J. Meinwald, personal communication). However, it is known that male *D. chrysippus* must spend several days collecting pheromone precursors (pyrrolizidine alkaloids) from plants; therefore those males able to maintain the highest foraging rates are likely to achieve earlier matings and greater overall success.

The major colour differences between the genotypes (see ref. 4, Figs 1–16) could influence metabolic rate and hence activity

levels. The amount of black and brown pigment is greatest in *aegyptius* and least in *dorippus* with most *transiens* intermediate. The darker coloration of *aegyptius* could allow it to maintain a higher body temperature, due to superior absorbance of radiant heat, during the cooler, cloudier months. It is interesting that *aegyptius* is the only form in the cooler subtropical parts of the species' range at both its northern and southern limits. Conversely, enhanced reflectance could provide relative protection for *dorippus* during the hot, dry season, a suggestion supported by the fact that it is the only form in the arid regions of northern Kenya and Somalia^{4,10}. The intermediate heterozygote could be better adapted than either homozygote to median radiation values and therefore better able to maintain optimum activity through the vicissitudes of a climate which can feature substantial wet or dry periods out of season, with successive years rarely having a consistent pattern.

These results are of interest in three respects: (1) they provide a rare¹¹ and unusually explicit example of heterozygous advantage at a single locus in a wild population. (Linkage disequilibrium involving the C and two other loci^{3,5} and epistasis⁴ implies that the real unit of selection is a supergene or inversion.) (2) The data show that heterozygous advantage is a mechanism capable of both conserving and releasing as required the genetic diversity on which selection can act cyclically in an oscillating climate. I emphasize here that the generation time of *D. chrysippus* is short in equatorial regions with 13–14 overlapping generations a year and that similar marked changes in morph frequency are known in other tropical species such as *Catopsilia florella*¹² and *Hypolimnas misippus*^{13,14}. (3) I conclude that male competition predominantly determines the outcome of sexual selection in *D. chrysippus* and outweighs female choice, receptivity or allure which are more often identified as critical factors in insects¹⁵.

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Table 2 Analysis of χ^2

Source of variation	χ^2	Degrees of freedom	Probability
Morphs × periods	124.16842	4	9.00×10^{-10}
Proportions mating × periods	14.66944	2	6.52×10^{-4}
Proportions mating × morphs	26.51889	2	1.74×10^{-6}
Residual heterogeneity	6.72306	4	0.151
Total	172.07981	12	

Table 3 Fitness of C-locus homozygotes relative to the heterozygote

Period	Condition	Genotypes			Total
		CC	Cc	cc	
April–August 1974	Mated	3	85	23	111
	Unmated	149	307	155	611
	Fitness	0.091	1	0.596	
September 1974–March 1975	Mated	59	76	2	137
	Unmated	209	196	47	452
	Fitness	0.788	1	0.146	
April–August 1975	Mated	5	61	26	92
	Unmated	114	197	145	456
	Fitness	0.178	1	0.643	

Genotype numbers are based on corrections for expressivity (see text).

Fluoromevalonate acts as an inhibitor of insect juvenile hormone biosynthesis

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The potential of analogues of insect juvenile hormones (JHs) for pest control has been demonstrated by the application of methoprene for the selective control of certain insects, including several mosquito species, the cigarette beetle and the hornfly¹. Compounds that impede JH production could offer a potentially more attractive method of control because accelerated metamorphosis would shorten the lifetime of the larvae, the stage that usually does maximum damage to the host plant. Of the few anti-juvenile hormone (AJH) agents described²⁻⁵, only precocenes evoke all the required biological responses, but they affect a few insect taxa of minor economic importance. Their activity is thought to result from cytotoxicity^{6,7} in the corpora allata. In view of similarities between the biosynthesis of the carbon skeletons of insect JHs^{8,9} and farnesyl pyrophosphate (a precursor of steroids and other terpenoids¹⁰), compounds that inhibit the early steps in the biosynthesis of cholesterol might be expected to suppress JHs. Although this concept has already been explored without success^{11,12}, we now report the inhibition of juvenile hormone biosynthesis (AJH activity, see Table 1 legend) by a fluorinated mevalonate (tetrahydro-4-fluoromethyl-4-hydroxy-2H-pyran-2-one, FMev, Fig. 1) which until now was known only to be hypocholesterolaemic¹³⁻¹⁵.

AJH activity was observed initially when 0-24-h third-instar larvae of the tobacco hornworm (*Manduca sexta*) were treated topically with 100 µg of FMev, which induced three types of effects, all directly related to JH deficiency. First, intense black pigmentation was often observed after the succeeding moult (to fourth instars). In *Manduca* this is known to result from JH deficiency^{16,17} and it therefore serves as a diagnostic tool in screening for JH suppressing activity. There were further signs of antagonism at the end of this instar, when many larvae started to show the prepupal burrowing behaviour normally occurring one instar later. In this case, the subsequent moult (to the fifth instar) often resulted in miniature pupae or intermediates between larvae and pupae with a varying mosaic of larval and pupal cuticle (Fig. 2). These morphologically premature pupae and intermediates were generally nonviable. Given higher doses, third-instar larvae started prepupal burrowing at the end of the third instar and produced pupal intermediates in the fourth instar. All these effects of FMev could be averted by concurrent application of a very active juvenoid such as hydro-prene; this 'rescue' confirms that the observed symptoms can be attributed to JH deficiency. A high dose of FMev (2.5 mg) injected into adult *M. sexta* 1-2 h after eclosion also prevented the maturation of eggs. This anti-gonadotropic activity is consistent with the expected properties of an AJH compound.

A considerable morphogenetic effect was observed after treatment of fifth-instar *Galleria mellonella* larvae with FMev. Untreated larvae normally developed through three additional instars before forming pupae approximately 15 mm long. Larvae treated with FMev produced premature pupae in any of these three subsequent instars, the smallest sixth-instar pupae being 4 mm long. Some of the large premature pupae survived to generate precocious miniature adults of otherwise normal appearance.

The AJH properties of FMev seem to be manifest only in lepidoptera. Larvae of the six moth species examined responded

Table 1 Anti-juvenile hormone* activity of FMev on several lepidopteran species

Insect	Instar treated	ED ₅₀ (mg per g)
<i>Manduca sexta</i> (tobacco hornworm)	Third	0.7
<i>Samia cynthia</i> (Cynthia moth)	Third	1.0
<i>Phryganidia californica</i> (California oak moth)	Third	1.0
<i>Galleria mellonella</i> (greater wax moth)	Fifth	40
<i>Spodoptera exigua</i> (beet armyworm)	Third	44
<i>Heliothis virescens</i> (tobacco budworm)	Third	> 200

0-24-h larvae of the indicated instar of each species were treated topically with 1 µl acetone dilutions of FMev and reared to the last larval instar. Observations were made throughout the bioassays. The ED₅₀ (effective dose causing 50% of the treated larvae not showing acute toxicity from FMev to demonstrate precocious metamorphosis or prepupal behaviour) is based on the mg mass of compound per g of insect. In the case of *H. virescens*, the ED₅₀ was never achieved, although a small percentage of the treated larvae did manifest premature pupation.

* We use the term 'anti-juvenile hormone (AJH) agents' to describe those chemicals which induce symptoms of JH deficiency, including premature metamorphosis. This general term can encompass the precocenes (cytotoxic to the corpora allata^{6,7}), FMev (inhibitor of JH biosynthesis) and AJH agents of as yet obscure³⁻⁵ mechanism of action.

in various degrees to the AJH effects of FMev (Table 1). No such effects were found when the larval stages of the following non-lepidopteran insects were treated with FMev: *Musca domestica* (housefly), *Aedes aegypti* (yellow fever mosquito), *Schistocerca nitens* (vagrant grasshopper), *Dermestes maculatus* (hide beetle), *Hypera postica* (alfalfa weevil) and *Oncopeltus fasciatus* (milkweed bug).

FMev exhibits moderate toxicity at high doses for lepidoptera which are less sensitive to the AJH effects of FMev. However, for more sensitive species such as *M. sexta*, toxicity is minor (5-10% at ED₅₀). Because this acute toxicity also occurs in insect species not responding with precocious metamorphosis and because concurrent application of a juvenoid did not rescue this mortality, we conclude that this toxicity is not related to inhibition of JH biosynthesis and results from another mechanism. A possible explanation for the nonspecific mortality is metabolic conversion of FMev to fluoroacetate, a potent biocide. Treatment of *M. sexta* larvae with ethyl fluoroacetate revealed substantial toxicity without AJH effects.

We originally tested FMev for AJH activity based on the rationale that it could serve as an antimetabolite of mevalonate and/or homomevalonate. Preliminary studies affirm that FMev

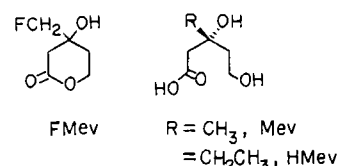


Fig. 1 Fluoromevalonolactone (FMev), mevalonate (Mev, precursor of cholesterol and insect juvenile hormones) and homomevalonate (HMeV, biosynthetic precursor of several insect juvenile hormones). FMev has been synthesized previously^{14,15}, but we synthesized it by treating ethyl fluoroacetate (highly toxic) with allylmagnesium bromide, followed by ozonolysis of the resultant diallylcarbinol from the Grignard reaction. The ozonide was reduced with sodium borohydride to give 3-fluoromethyl-1,3,5-pentanetriol, which was selectively oxidized to FMev with chromium trioxide in aqueous sulphuric acid (Jones reagent).

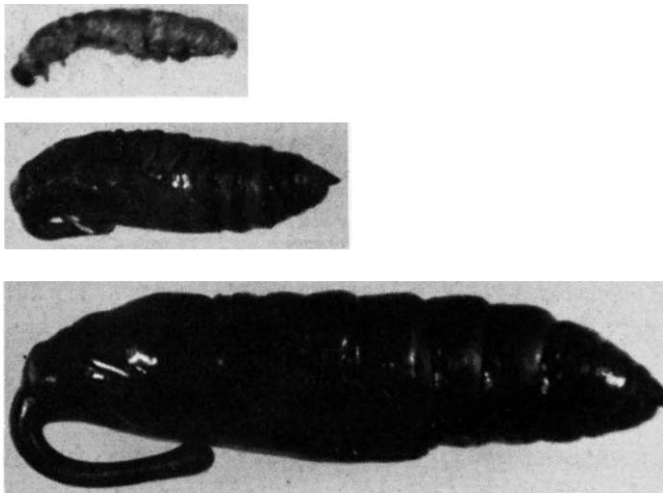


Fig. 2 A larval-pupal intermediate, precocious pupa and normal pupa (from top to bottom) for *Manduca sexta* (tobacco hornworm). The accelerated metamorphosis for the intermediate and precocious pupa was induced by treating third-instar larvae with 100 µg of FMeV.

inhibits the biosynthesis of insect JHs. The titre of JHs in *M. sexta* haemolymph is markedly reduced when larvae are treated topically with the physiologically effective dose of FMeV (J. P. Edwards and B. J. Bergot, unpublished). An *in vitro* study with homogenates of *M. sexta* corpora allata showed significant inhibition of metabolism of ^{14}C -mevalonate and ^3H -homomevalonate to subsequent precursors of JHs (F. C. Baker, unpublished).

We have synthesized many analogues of FMeV, mevalonolactone and related compounds. Five of them show AJH activity on lepidopteran larvae, but are less active than FMeV. Four of these marginally active compounds are likely to be active because of their facile metabolism to FMeV (for example, 3-fluoromethyl-1,3,5-pentanetriol). The difluoromethyl analogue of FMeV was five times less active on *M. sexta* than FMeV, whereas the trifluoromethyl analogue was devoid of AJH properties.

We have thus found that a compound which is hypocholesterolaemic may also have AJH activity; perhaps other hypocholesterolaemic agents may serve as chemical structures which can be manipulated by analogue synthesis to optimize AJH activity. However, tests of several chemically unrelated materials with similar hypocholesterolaemic activity have revealed no other active inhibitors (ref. 12 and G.B.Q. *et al.* unpublished).

The activity of FMeV by itself is not sufficiently high, even on lepidopteran pests, for commercial exploitation. Nevertheless, FMeV should be an invaluable tool in studies of lepidopteran physiology and endocrinology. We hope the novel mode of action—inhibition of JH biosynthesis—can form the basis for new selective insect-control agents.

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A protein called immaturin controlling sexual immaturity in *Paramecium*

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As in many metazoans, clones of some species of *Paramecium* have, after conjugation, a period of immaturity during which the cells cannot mate. The duration of immaturity is measured by the number of cell generations, which remains fairly constant, although duration in time varies with rate of cell reproduction^{1–3}. Genic involvement is shown by mutants with reduced periods of immaturity⁴. In three different groups of *Paramecium* species, the cytoplasm of immature cells apparently contains the same substance which represses mating activity when injected into sexually mature cells^{5–7}. The immaturity-inducing substance seems to be absent from sexually mature cells, as brei made not only from mature cells in the stationary phase (mating-reactive cells), but also from those in the log phase (mating-non-reactive cells), does not repress mating activity when injected into mature cells⁵. Variations in the amount of the substance during immaturity suggest that it controls the duration of the period⁸. We have isolated and partially characterized the substance—a single protein called immaturin. The activity of immaturin is dose dependent and associated with a heat-labile protein of molecular weight (MW) 10,000.

For quantitative assay of immaturin activity by microinjection, we grew each injected cell in a glass capillary (0.6 mm inside diameter, 75 mm long) containing 20 µl fresh lettuce-juice medium inoculated with a non-pathogenic strain of *Klebsiella pneumoniae* 1 day before use⁹. The cell in each capillary produces exactly 16 cells which then enter synchronously into stationary phase. The 16 cells are isolated and then provided with mating-reactive cells of complementary mating type. If they are mature, at least 13 of the 16 form mating clumps with the cells of complementary type (each clump consisting of one cell of the type being tested, and a group of reactive cells of the opposite type). The activity of injected immaturin was measured by the percentage of sets of 16 forming less than 13 clumps (the clumps were usually from 10 to 0 when the injection was effective).

The immaturity period of the strains of *Paramecium caudatum* we used was about 50 fissions after conjugation. For isolation of immaturin, cells ($\sim 9 \times 10^7$), about 20 fissions after conjugation, were washed with solution A (10 mM KCl, 5 mM MgCl_2 , 10 mM Tris-HCl, pH 7.4) and then with solution A' (solution A + 0.25 M sucrose) by slow centrifugation (100–200g). The pellet was homogenized with an injection syringe¹⁰ and the brei thus obtained was centrifuged at 105,000g for 60 min at 2°C. The supernatant was filtered through a 0.45-µm Millipore filter. The filtrate was applied to a Sephadex G-50 column and each fraction was assayed by injecting 15-µl samples into mature cells. When the most active fraction (no. 32) was injected, 16 clones out of 20 formed less than 13 clumps when tested. Among those 16 clones, 4 formed no clumps and 10 formed less than 8 clumps. When the same fraction from mature cells was injected, 40 out of 51 formed 16 clumps and the remaining 11 formed more than 12 clumps. Then, the active fractions (nos 31, 32 and 33 in Fig. 1) were chromatographed on a DEAE-Sephadex A-25 column. The activity of immaturin was eluted as a single peak at about 400 mM NaCl (Figs 1, 2). In the test of the peak fraction (no. 10), 15 clones out of 19 injected formed less than 13 clumps. Among those 15, 10 formed less

Table 1 Purification of immaturin

Step	Total protein obtained (mg)	No. of cells injected	Activity (%)	Protein injected per cell (pg)	Specific activity
(1) 105,000g supernatant	154.4	22	72.7	15	4.8
(2) Sephadex G-50 chromatography	3.6	25	88.0	0.7	125.7
(3) DEAE-Sephadex chromatography	0.013	25	80	0.03	2666.6

Activity is % of mature clones which contained more than 25% immature cells after injection. Specific activity is defined as activity per pg injected protein. Amount of protein was determined by the method of Lowry *et al.*²¹.

than 9 clumps. The degree of purification was calculated on the basis of the activity of the 105,000g supernatant.

The relation between concentration of immaturin and activity of each concentration was first examined by injecting a dilution series of the supernatant. The activity (% of immaturity brought about by injection) was nearly proportional to the amount of protein injected. When specific activity was defined as activity per pg injected protein, three purification steps increased the specific activity of immaturin about 550-fold (Table 1). To determine the purity of each fraction, samples from the DEAE fractions were subjected to acrylamide-gel electrophoresis by a modified method of Davis¹¹. Lyophilized material from the peak fraction (no. 10) of the DEAE-Sephadex elution was dissolved in 300 μ l of solution A and 100- μ l samples were applied to a gel containing 11.25% acrylamide. Only a single band was strongly stained with Coomassie brilliant blue, and no minor band was detected (Fig. 3).

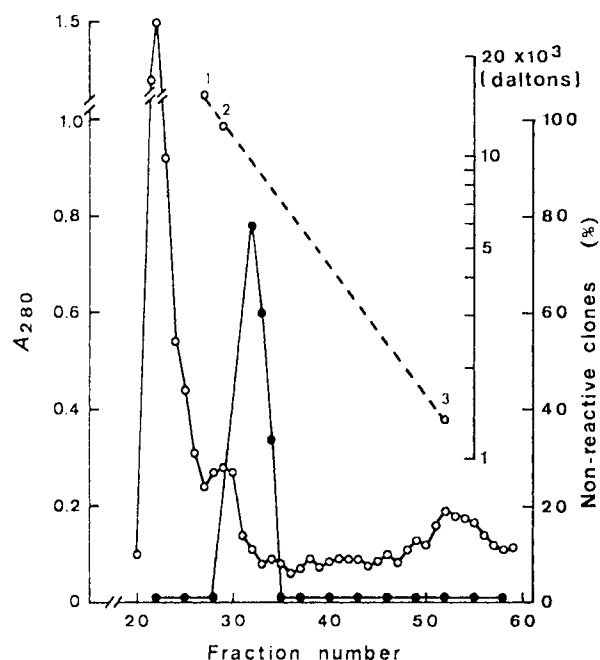


Fig. 1 *a*, Sephadex G-50 elution profile of the soluble fraction of sexually immature cells. A 2.5-ml sample of the supernatant obtained by homogenization and 105,000g centrifugation of immature cells (9×10^7) was applied to a Sephadex G-50 (Pharmacia, fine) column (2.5×70 cm) and chromatographed at 5°C. Each fraction (4.4 ml) was assayed by injecting 15- μ l samples into mature cells. $\circ$, 280-nm absorbance; $\bullet$, activity of immaturin (% of non-reactive clones brought about by the injection). Broken line, calibration curve of MWs: 1, myoglobin, 18,500; 2, cytochrome *c*, 13,000; 3, cyanocobalamin, 1,355 (all Sigma). *b*, DEAE elution profile. Active fractions (nos 31–33) from the Sephadex G-50 filtration were applied on a DEAE-Sephadex A-25 column (0.9×15 cm) containing 4 ml gel. Before application of the sample, the column was equilibrated with 50 ml solution A (see text). The elution medium was a linear gradient of from 0 to 500 mM NaCl in solution A. Size of each fraction was 4.4 ml $\circ$, 280-nm absorbance; $\bullet$, activity; broken line, concentration of NaCl.

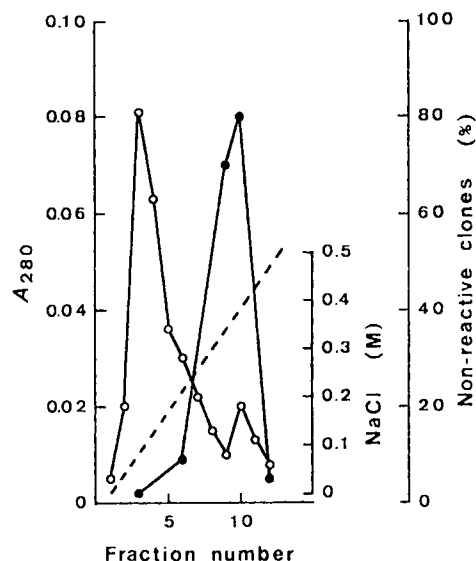


Fig. 2 Polyacrylamide-gel pattern of the peak fraction of the DEAE chromatography (see text for details).

The MW of immaturin was estimated to be about 10,000 from Sephadex G-50 chromatography by comparing with marker molecules (cyanocobalamin, cytochrome *c* and myoglobin, Fig. 1). Determination of the MW using SDS-polyacrylamide gel electrophoresis was unsuccessful because the standard curve of MWs <11,000 was not linear. Immaturin lost its activity when exposed to polyacrylamide gel-bound insoluble trypsin (Sigma) for 65 min at 27–28°C, whereas no loss of activity was observed when exposed to heat-inactivated, insoluble trypsin or agarose-bound, insoluble ribonuclease (Sigma) (Table 2). The activity was completely destroyed when exposed to 100°C for 6 min.

Table 2 Activity of immaturin when treated with enzymes and heat

Treatment	No. of cells injected	No. of non-reactive clones	Activity (%)	Confidence interval (90%)
None	28	22	78.6	62–92
Trypsin	28	1	3.4	0.1–14
Heated trypsin	25	17	68.0	48–84
Ribonuclease	26	14	53.8	36–72
100°C, 6 min	26	0	0	0–10.2
No injection	30	0	0	0–10

Insoluble trypsin (2.5 U, Sigma) or insoluble ribonuclease (5 U, Sigma) was added to fraction no. 9 from the DEAE-Sephadex column, in a solution containing 10 mM KCl, 5 mM MgCl₂ and 10 mM Tris-HCl (pH 7.4), and incubated in a waterbath with shaking at 27–28°C for 65 min. The particle-bound enzymes were removed by centrifugation at about 1,000g for 5 min and the supernatants were assayed for immaturin activity by injecting about 15 μ l into mature cells. Heated trypsin was obtained by heating at 100°C for 6 min. Activity is per cent of mature clones which contained more than 25% immature cells after injection (here called non-reactive clones).

From these results we conclude that immaturin is a heat-labile protein, which we isolated almost free of other proteins. We did not rigorously test the sample for purity from organic substances other than protein, but the purification procedure described above probably removed most of them. As repeated washing by ultrafiltration through a Diaflo membrane UM2 (Amico) at 4 °C under a pressure (2.0 kg cm⁻²) of nitrogen gas did not decrease the activity of immaturin, it is unlikely that small molecules attached to protein have the immaturin activity. This experiment also seems to eliminate the possibility that loosely bound, small molecules are cofactors of immaturin, though it does not completely eliminate the possibility that tightly bound small molecules are cofactors or the site of immaturin activity.

Mating ability in *Paramecium* depends primarily on the presence of sexual cell-recognition molecules, located on the ciliary membranes and called the mating substances^{12,13}.

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Specificity of the mating substances is known to be controlled genetically^{9,14,15}. Immaturin evidently inhibits the expression of mating substance activity but the mechanism of its action, or its target of action within a cell, remains to be investigated. Immaturin may be comparable to several factors controlling gene expression, such as insulin and steroid hormone receptors in higher animals^{16,17}, repressors in *Escherichia coli*¹⁸, CGA of *E. coli*¹⁹, and the inhibitor of rRNA synthesis in *Xenopus*²⁰. Isolation and characterization of immaturin should facilitate study of mechanisms of genic control of mating ability in *Paramecium*, of genic regulation of life-cycle phases in clonal ageing, and of cell differentiation in general.

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Lyt 1⁺23⁻ cells appear in the thymus before Lyt 123⁺ cells

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Most thymocytes are either immature or functionally deficient and express a series of lymphocyte cell-surface antigen markers designated Lyt 1, Lyt 2 and Lyt 3 (refs 1, 2) which have been useful in distinguishing functional subsets of T cells^{3,4}. In contrast, a small population of cortisone-resistant thymocytes (CRT), confined to the thymic medulla after acute corticosteroid treatment are functionally more mature. These cells, like peripheral T cells, have restricted expression of Lyt antigens and mostly are either Lyt 1 or Lyt 123 cells³⁻⁵. It has thus been assumed that all thymocytes initially are Lyt 1⁺, 2⁺, 3⁺ and by differentiation lose either Lyt 1 or Lyt 2, 3 to result in Lyt 1⁺(23⁻) and Lyt 1⁻(23⁺) cells⁶⁻⁸. Using immunofluorescence (IF) and flow microfluorometry (FMF) analyses to detect Lyt antigen expression quantitatively without the requirement for cell lysis, we have now re-examined the expression of Lyt 1, 2 and 3 antigens on murine fetal thymocytes from 14 to 19 days of gestation and on normal thymocytes from birth to 2–3-month-old adults. These studies demonstrate that cells with the Lyt 1⁺23⁻ phenotype first appear in the thymus several days before Lyt 123⁺ thymocytes are detected, and suggest either a micro-environmental or site-specific influence for phenotypic differentiation and/or two independent, pre-committed lineages.

The details of the methods used for FMF detection of IF for Lyt have been reported previously⁵. For these studies, in addition to alloantisera prepared in our laboratory, monoclonal reagents for Lyt 1.1 and Lyt 2.2 specificities have been used to

avoid any problems of nonspecific reactions. In our experience, these monoclonal reagents give patterns of reactivity both in adult lymphoid tissue distribution and strain distribution equivalent to the related, allelically specific antisera described by others⁹.

Data from one experiment are shown in Table 1. With fetal thymocytes from C57BL/6-Ly-1^a mice, an increased proportion of Lyt 1⁺ thymocytes are observed between 15 and 19 days of gestation. Using monoclonal anti-Thy 1.2, 80% of the fetal thymocytes are positive for Thy 1 expression at the 15 and 16 day points, whereas >97% are positive for Thy 1 at later time points (data not shown). These positive cells also express quantitatively increased amounts of Lyt 1 antigen. This latter conclusion is drawn from the increase in median fluorescence units (FU) for the thymocytes at each time point and can be seen more explicitly in data from an additional experiment in Fig. 1. This increase in the level of Lyt 1 expression per cell occurs despite the fact that cell size is decreasing through this period, as reflected by the decrease in the median light scatter units. For comparison, these same parameters are shown for adult thymocytes. Thus, in contrast to adult thymocytes¹⁰, the quantitative level of the Lyt 1 phenotype is not related to cell size.

Until day 17 of gestation, almost no cells expressing Lyt 2 or Lyt 3 antigen are seen (Table 1). Figure 2 shows the IF profiles of Lyt 3.2 expression on thymocytes from embryos at days 16 and 17 of gestation. In a series of six experiments, a marked increase in the proportion of thymocytes expressing both Lyt 2 and Lyt 3 is seen (7–15% above control background on days 14–16 to >70% on day 17). The adult phenotype, 10% Lyt 1⁺23⁻:90% Lyt 123⁺ which we previously described⁵, is observed at 18 days, about 24 h before birth in this strain. At no time are all the fetal thymocytes Lyt 123⁺.

The dramatic appearance of the Lyt 123⁺ cells on day 17 of gestation follows differentiation of cortical histological sites in the murine thymus observed by others at 15 days of gestation^{11,12}. The appearance of these cells also corresponds to a marked increase in cell number which occurs at this time. These data suggest either that Lyt 123⁺ cells are derived from Lyt 1⁺23⁻ precursors, or that two separate pre-committed lineages for Lyt 1⁺23⁻ and Lyt 123⁺ cells in the thymus take up residence at different times during ontogeny. The concept of two independent lineages of cells, either or both of which may

Table 1 Lyt expression on fetal thymocytes

Fetal age (days)	Lyt 1		Lyt 2 % positive	Lyt 3 % positive	Median LSU of viable cells	% Large cells	No. of cells per thymus
	Minimum % positive	Median FU of positive cells					
15	50.1	201 (77)	6	ND	606	70	4×10^5
16	51.8	236 (69)	15	13	600	68	5.7×10^5
17	95.8	375 (42)	72	73	564	63	3.3×10^6
19	96.5	365 (39)	79	80	516	52	4×10^6
Adult	98.4	439 (31)	91	91	440	21	$1-2 \times 10^8$

Fetal age was determined by calculating from the date of detection of a vaginal plug (plug date = day 0). Minimum % positive was determined by the number of stained cells above the intersection of the IF profile of anti-Lyt 1.1 plus fluoresceinated goat anti-mouse immunoglobulin (FIGAMiG) stained cells and the IF profile of the cells treated with FIGAMiG alone. This type of intersection is seen in Fig. 1. The median values for Lyt 1⁺ cells are calculated above the intersection (145 FU) for this experiment. FU are calculated as previously described¹⁰. For comparison the value for surface immunoglobulin on B cells is ~6,000 FU. Median FU for the controls are given in parentheses next to the FU values for Lyt 1⁺ cells. Light scatter units (LSU) are arbitrary units of measurement reflecting cell size which have been described by others and which we have used previously for comparison of thymocytes^{5,27}. Large thymocytes are reduced to about 20% of the total thymocyte population within 1 week of birth. The number of cells per fetal thymus was determined from the total cell recovery of 7-12 embryos at each time point. ND, not determined.

subsequently be subdivided, is certainly not a new proposal for thymocyte differentiation. However, the characterization of these two lineages with different thymocyte phenotypes in the fetal development of the thymus has not been demonstrated previously.

This finding that Lyt 1⁺23⁻ cells are present in the fetal thymus before Lyt 123⁺ cells contrasts with the previously proposed concept that all thymocytes are Lyt 123⁺ before differentiative loss of Lyt 1 or Lyt 2 and Lyt 3 (ref. 4). After birth, CRT³⁻⁵, the thymus migrant population¹³ and thymocytes

non-agglutinated with peanut lectin¹⁰ all contain both Lyt 1⁺23⁻ and Lyt 123⁺ cells, presumably in a more mature state of differentiation. In normal adult thymocytes, Lyt 1⁺23⁺ cells are not seen by IF with the quantitative methods used here^{5,10}. Therefore, if one still wishes to assume that all thymocytes and T cells differentiate through an Lyt 123⁺ state, it is required that at least during ontogeny Lyt 1⁺23⁻ cells generate Lyt 123⁺ cells, and from those, more mature Lyt 1⁺23⁻ cells and Lyt 123⁺ cells which exit from the thymus. These data do not exclude the possibility that some Lyt null precursors express all three Lyt antigens simultaneously, nor do they exclude the possibility that a small number of Lyt 123⁺ precursors are instantly driven to Lyt 1⁺23⁻ in early ontogeny.

The fetal thymus of the mouse develops in a particular histological pattern of differentiation. Initially, the thymic rudiment appears as a largely epithelial structure with a sparse lymphoid population, comparable in both lymphoid type and size to the medullary area of the differentiated adult thymus. Subsequently, the densely lymphoid cortical areas which are largely composed of small thymocytes appear and a particular histological type of cortical epithelial cell develops in the fetal thymus^{11,12,14}. It is almost certain that some stem cells in the early fetal thymus are capable of differentiating into Lyt 123⁺ cells, because Owen and Ritter¹⁴, Mandel and Russell^{11,12}, and Kamarck and Gottlieb⁸ have observed small cortical thymocyte differentiation in murine fetal thymus organ cultures deprived of traffic of additional cells from the periphery. Furthermore, Kamarck and Gottlieb⁸ indicated that Lyt 123⁺ cells could differentiate in these fetal organ cultures. This is being tested further with fetal thymus transplants, which are derived from mice expressing allelically different Lyt specificities.

Thus, the microenvironment of the thymus may be critical for phenotype generation. When the early 'medullary' epithelial environment is predominant, perhaps only Lyt 1⁺23⁻ cells can develop. Once the cortical epithelial cells^{11,12,14} have differentiated an appropriate site for Lyt 2, 3 expression, these antigens may then be detected on populations subsequently differentiating in the thymus. This would presume a common precursor with no site-specific homing, but site-specific differentiation would predict that adult thymic repopulation studies would show the adult Lyt phenotypes and not the sequential pattern seen in ontogeny.

The alternative possibility of two separate lineages would more easily explain both the finding that there are, from an early time point in ontogeny, Lyt 1⁺23⁻ thymocytes as demonstrated here, and that a small proportion of thymocytes with this phenotype are found throughout adult life⁵. This could also explain other phenotypic subset analyses of the differential expression of Thy 1 in the thymus¹⁵, as well as sequential reactivity of fetal thymocytes to phytohaemagglutinin and concanavalin A¹⁶.

Avian thymus studies indicate that the differentiation involves two lineages which may even have different pre-thymic path-

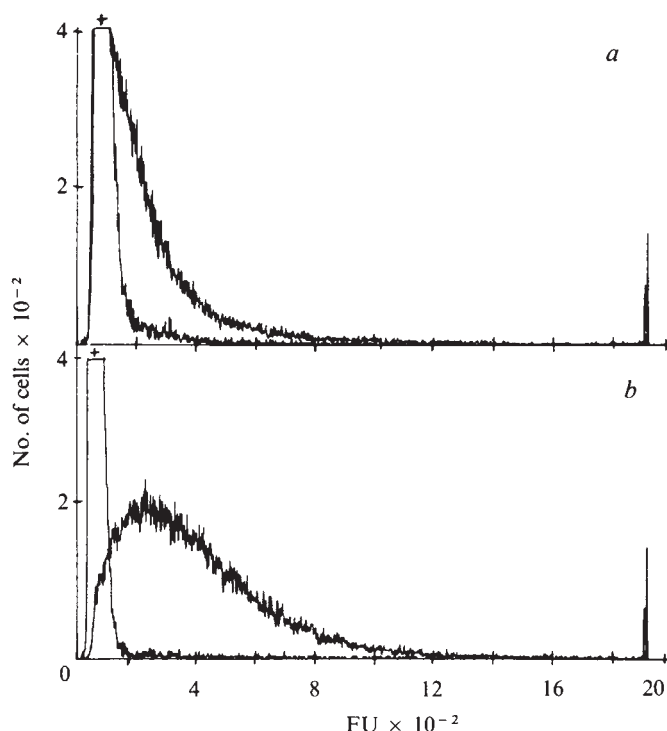


Fig. 1 Fetal thymocytes from B6-Ly 1^a mice which express the Lyt 1.1 antigen were treated with anti-Lyt 1.1 monoclonal antibody (clone line 39/107) and FIGAMiG. The details of the fluorescent reagents and the IF procedure are published elsewhere⁵ as are the details of the use of the FACS-II (Becton Dickinson) for quantitative FMF analysis^{5,27}. The data configurations presented in *a* and *b* as the IF profiles on 50,000 cells per sample are also described elsewhere²⁸. In *a*, 15-day fetal thymocytes, the positive cells (cells with >160 FU), are compared with negative control fetal thymocytes from B6 mice which express the Lyt 1.2 antigen. As seen in *b*, the fetal thymocytes at day 17 of gestation are much more positive, with a reduced overlap between the positive cells and the negative control, in this case FIGAMiG treatment alone.

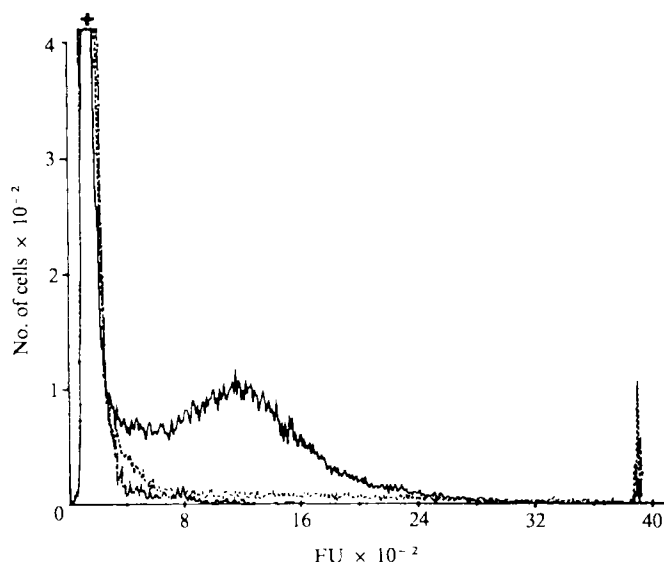


Fig. 2 Fetal thymocyte from B6-Ly-1^m mice which express the Lyt 3.2 antigen were treated with anti-Lyt 3.2 serum⁹ and FIGAMig antibodies. At 17 days gestation (—), the IF is seen as a peak of positive cells whereas at day 16 (---) the IF is less than 10% above the 16-day fetal thymocytes treated with FIGAMig (---) alone. The median value of the Lyt 3⁺ cells was not noticeably changed in these experiments and varied only between 1,200 and 1,600 FU. The FIGAMig control has 3.8% cells >400 FU and 1.6% of cells >800 FU.

ways^{17,18}. An early cohort of thymic cells passes through the bursa of Fabricius, a B-cell differentiation site in birds, and takes up residence in the thymus. Cells, which populate thymus at a later time in development, apparently bypass the bursa. This parallels the two-stage distinct sequential appearance seen for the two Lyt phenotypes reported here. One might even consider that precursors of Lyt 1⁺23⁺ cells pass some similar B-cell site at which a T-helper function is in some way induced or programmed in the lineage. This is clearly hypothetical in the mouse, because no comparable unified B-cell generation site is known. However, there is evidence of separated functions of isolated subpopulations of Lyt differentiated peripheral T cells¹⁹.

Finally, a combination model is proposed in which Lyt 1⁺23⁺ cells give rise to two separate lineages only one of which 'seeks' a cortical site or pathway. This Lyt 123⁺ lineage may require multiple divisions in the cortex for both functional and phenotypic differentiation and for repertoire development. The requirement for cell division has been documented in other differentiation systems²⁰. This model of separate lines with site-specific differentiation would be consistent with data and models of thymocyte differentiation presented by several investigators, including Weissman²¹, Shortman and Jackson¹⁵, Zinkernagel²² and Stutman²³. Although Leckband and Boyse²⁴ previously indicated a correlation with TL⁺ phenotype of CRT, a presumably more mature medullary thymic subpopulation, recent observations of Kisielow *et al.*²⁵ show TL⁺ cells with differentiated Lyt phenotypes. Data obtained from analysis of thymic lymphomas²⁶ led us to propose earlier that such immature thymocytes might exist which were TL⁺ but differentiated for Lyt phenotype. Thus, we favour this latter combination model which presumes independent pre-committed lineages with site-specific differentiation and therefore presumes nothing about the maturity of cells found in either the cortical or medullary site in the thymus.

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Selective inhibition of T suppressor-cell function by a monosaccharide

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Interactions between regulatory T lymphocytes and other cells are assumed to occur at the level of the cell surface. T cells which suppress the generation of specifically effector cells have been described as having antigenic, idiotypic, allotypic and I-region specificity^{1–4}. Other T suppressor cells generated by *in vitro* cultivation with or without mitogenic stimulation^{5,6} have suppressive activity for T and B cells but no specificity can be assigned to them. These T suppressor cells (T_s) inhibit various lymphoid functions—this either reflects their polyclonal origin or indicates that the structures recognized by the T_s receptors must be common for many cell types. Carbohydrates on cell membrane-inserted glycoproteins or glycolipids might function as specific ligands for recognition by cellular receptors or soluble factors. Almost all cell-surface proteins of mammalian cells are glycosylated. There is evidence for lectin-like carbohydrate binding proteins not only in plants⁷ but also in toxins⁸, viruses⁹, prokaryotic cells¹⁰ and even mammalian cells, including T cells¹¹. A functional role for these lectin-like proteins has been described for slime moulds and suggested for the selective association of embryonic cells^{12,13}. We report here that addition of a monosaccharide can counteract the effect of T suppressor cells during the generation of alloreactive cytotoxic T cells (CTLs) *in vitro*.

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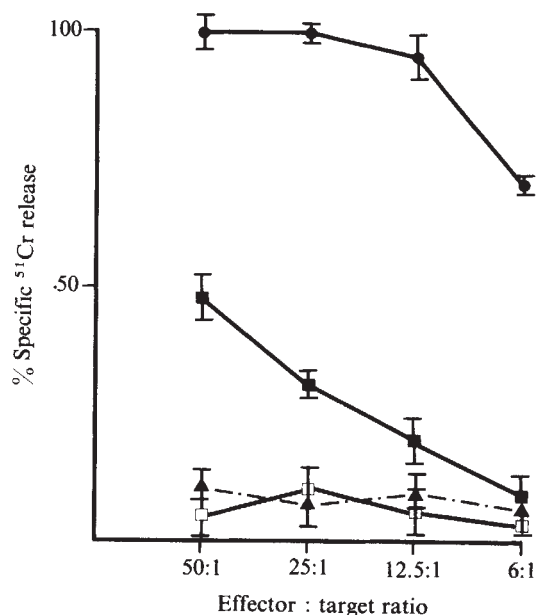


Fig. 1 Regulatory effects of MM on suppressor-cell function. Alloreactive CTLs were generated from C57BL/6J responder spleen cells by incubation with 2,000 rad-irradiated allogeneic DBA/2J spleen cells (6–8 week-old mice from the Institut für Versuchstierzucht, Hannover). Cells were cultivated in 24 flat-bottom Linbro plates (Flow) at a responder/alloantigen ratio of 1:1 and a concentration of 2.5×10^6 responder cells per ml RPMI medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Cells were collected 6 days later and tested for cytolytic activity in the ^{51}Cr -release assay¹⁷ using P-815 mastocytoma cells as targets. ●, Suppressor cells (T_s) from C57BL/6J mice were generated by precultivation in the absence of alloantigens using the method of Rollwagen and Stutman¹⁵ with the modification that erythrocyte lysis was omitted. When tested for cytolytic activity after 6 days, these cells were found to be negative (▲). After addition of these precultivated cells to new cultures containing responder cells and alloantigens at the ratio of responder/ T_s varying from 1:2 to 1:8 (this experiment 1:4), production of alloreactive CTLs was abolished (□). Addition of 10 mg ml^{-1} MM (Roth; Sigma; Calbiochem; Hoechst) counteracted the suppressive effect (■). Experiments were carried out in triplicate. Bars indicate standard deviations.

Nonspecific suppressor-cell production in *in vitro* cultivated lymphocytes by the plant lectin concanavalin A (Con A) can be inhibited by the addition of methyl- α -D-mannopyranoside (MM) during the induction phase¹⁴. We wondered whether similar phenomena could be demonstrated in a system without Con A. T_s were produced *in vitro* in cultures of spleen lymphocytes incubated in complete medium without antigenic stimulation for 6 days, as described elsewhere¹⁵. Suppressor cells produced in this way inhibit a variety of immune responses nonspecifically and without H-2 restriction. When added in graded numbers to a primary mixed leukocyte culture (MLC) on day 0, these cells were able to suppress or abolish the generation of alloreactive CTLs. This inhibitory activity was neither due to cell crowding effects (data not shown) nor to cytolytic activity in the suppressor cell populations¹⁶. Data from a representative experiment showed that generation of CTLs is abolished in the presence of suppressor cells (Fig. 1) and that these cells were not cytolytic after cultivation with antigen. Addition of 10 mg per ml MM to the culture system counteracted the suppressive effect, resulting in the reconstitution of a cytolytic response.

Treatment of the transferred cells with anti-Thy-1.2 and complement abolished their suppressive effects, demonstrating that the MM-sensitive suppression is a T cell-dependent phenomenon (Fig. 2).

Table 1 Ability of methyl- α -D-mannopyranoside (MM) to inhibit the function of suppressor cells

Molar concentration of sugar during CTL induction	Ratio of responder: suppressor cells	Ratio of killer: target cells required for 1 LU	r^2	% T_s activity
—	1:0	10.0	0.97	0
—	2:1	39.1	0.95	100
10^{-2}	2:1	15.8	0.99	20
10^{-3}	2:1	17.2	0.98	25
10^{-4}	2:1	18.6	1.00	30
10^{-5}	2:1	27.2	0.97	59

Methyl- α -D-mannopyranoside (Roth) was re-crystallized and added at different molar concentrations to the culture containing responder cells, allogeneic stimulator cells and suppressor cells. Effector cells produced in these conditions were tested for cytolytic activity at 4 effector: target cell ratios (third column). The killer: target cell ratio required for 1 lytic unit (LU), arbitrarily defined as the ratio resulting in 33% specific lysis in the 4-h assay period, was calculated by logarithmic curve fitting¹⁷. r , Coefficient of determination. The cytolytic capacity of suppressed responder-cell cultures was taken as 100% T_s activity.

Addition of the carbohydrate alone to the culture medium did not inhibit the generation of alloreactive CTLs. MM therefore does not interfere with antigen recognition, differentiation and expansion of alloantigen-specific T cells (Fig. 3a). Furthermore, no inhibition by MM was seen in the effector phase of cytolytic T

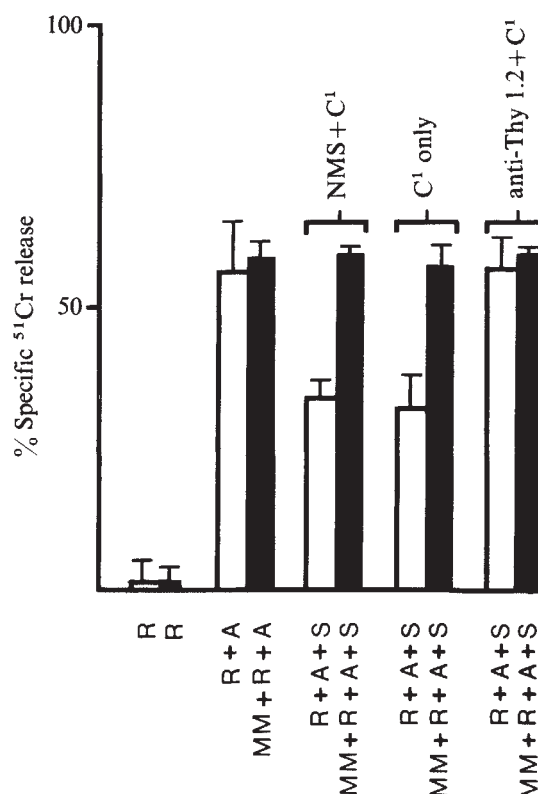


Fig. 2 Characterization of the MM-susceptible suppressor cells. Generation of alloreactive CTLs in presence or absence of MM and suppressor cells. After the 6-days induction phase, suppressor cells were incubated with either normal mouse serum (NMS) or anti-Thy-1.2 serum (1:20 final dilution) for 30 min at 20°C , centrifuged and resuspended in freshly thawed selected rabbit serum as a source of complement. Further incubation was at 37°C for 30 min. R, C57BL/6J responder cells ($2.5 \times 10^6 \text{ ml}^{-1}$); A, DBA/2J allogeneic cells ($2.5 \times 10^6 \text{ ml}^{-1}$); S, suppressor cells ($0.6 \times 10^6 \text{ ml}^{-1}$); MM, methyl- α -D-mannopyranoside (10 mg ml^{-1}). Effector: target cell ratio, 25:1; target, P-815 mastocytoma cells.

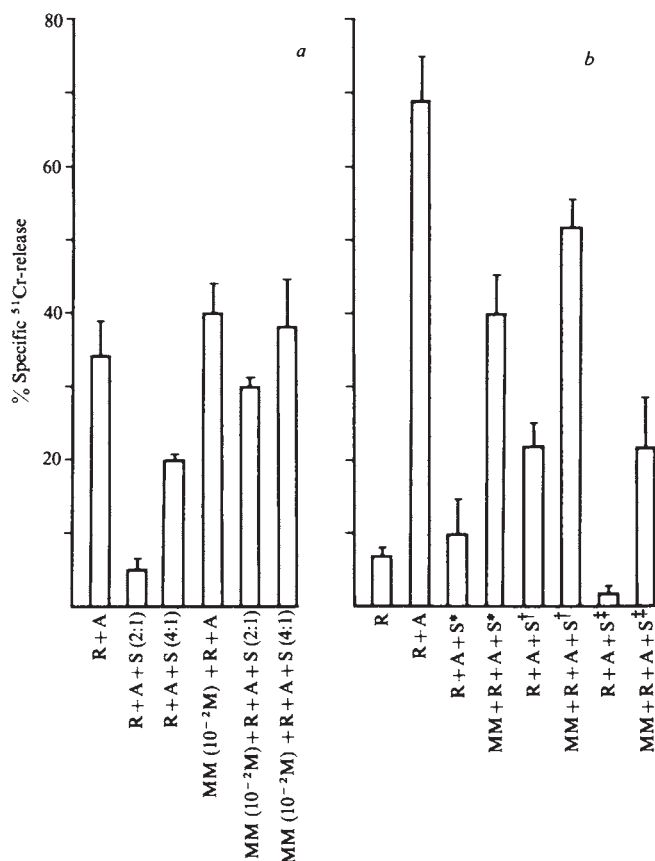


Fig. 3 Effect of MM on the generation of effector T cells. *a*, Effect on killer-cell generation. Responder cells (R) in presence of allogeneic cells (A) (see Fig. 1 legend) generate CTLs (first column). This effector-cell generation is suppressed in presence of suppressor cells (S) added at different R:S ratios at the start of culture. Addition of MM (10^{-2} M) inhibits suppressor-cell function (columns 5, 6) but not the generation of CTLs in the absence of suppressor-cells (column 4). *b*, Effect on suppressor-cell generation. * Suppressor cells (S) were generated as described in Fig. 1 legend and added to the culture to suppress CTL generation in presence or absence of 10 mg per ml MM. † Suppressor cells were cultivated for the first 72 h of the 6-day induction period in the presence of MM (10 mg ml^{-1}) and then tested for function in presence or absence of MM. ‡ Suppressor-cell generation in presence of MM during the 6-day induction period. Effector/target cell ratio in both experiments, 50:1; responder/suppressor cell ratio in *b*, 4:1.

cells because target cell lysis was the same in the presence or absence of MM (10 mg ml^{-1}) (data not shown). Similarly MM did not affect the induction phase of suppressor-cell generation (Fig. 3*b*). Spleen cells were cultivated in presence of MM either for the initial 72 h or the whole 6-day induction period before addition to the second culture. Both populations were found to have comparable suppressive activity when transferred to the MLC. This indicates that MM does not affect the generation of T_s from their precursors. Furthermore, we found no evidence for reduced DNA synthesis (^3H -thymidine incorporation) or decreased viability of T_s in culture in the presence of MM (data not shown). However, the capacity of the suppressor cells to inhibit a primary CTL was inhibited when MM was added to the second culture (Fig. 3*b*). Due to the different susceptibility to carbohydrate in the culture medium, our results clearly define cytotoxicity and suppression in this system as two separable manifestations of effector T-cell functions, a fact which is only shown with difficulty during regulation of CTL generation by T_s .

The effect of MM on T_s effector function could be attributed to the carbohydrate and not to impurities of the reagent used

because MM from different sources (data not shown) and also the re-crystallized sugar were equally effective (Table 1). Osmotic effects seem unlikely because several other monosaccharides were ineffective (Fig. 4). A toxic effect of MM on lymphocytes is also unlikely because concentrations even 100-fold higher than those active in our system were not toxic to leukocyte function¹⁹.

Our study demonstrates that cell interactions can be blocked by monosaccharides. Only the regulatory function of T_s is counteracted—neither CTL effector function nor the generation of T_s and CTLs is inhibited. At this stage it is impossible to determine how the T_s interact with their targets during CTL activation. Alternative mechanisms must be considered. The sugars may sterically interfere with structures involved in recognition of target cells by T_s . MM could also have reversible selective inhibitory effects on T_s metabolism, affecting function but not differentiation, perhaps by interfering with the glycosylation of recognition structures. We favour the hypothesis that recognition structures on T_s or secreted T_s products, by interaction with complementary structures on their target cells, mediate suppression and that the carbohydrate competitively blocks this interaction. Results obtained with stem cells²⁰ and T cells²¹ suggest that differentiation may be accompanied by a change in carbohydrate binding capacity of cell-surface components.

The fact that D-mannose is often found in mammalian glycoproteins either as core or end groups²² does not mean that the specificity resides only in this sugar. Other mono-, and in particular, oligosaccharides should be tested for specificity by determining the concentration required to inhibit the regulative T-cell interaction. The advantage of our system is that it studies functional interactions. However, one disadvantage is that the incorporation of sugar into cells may decrease their capacity to compete at the extracellular level and the concentration of

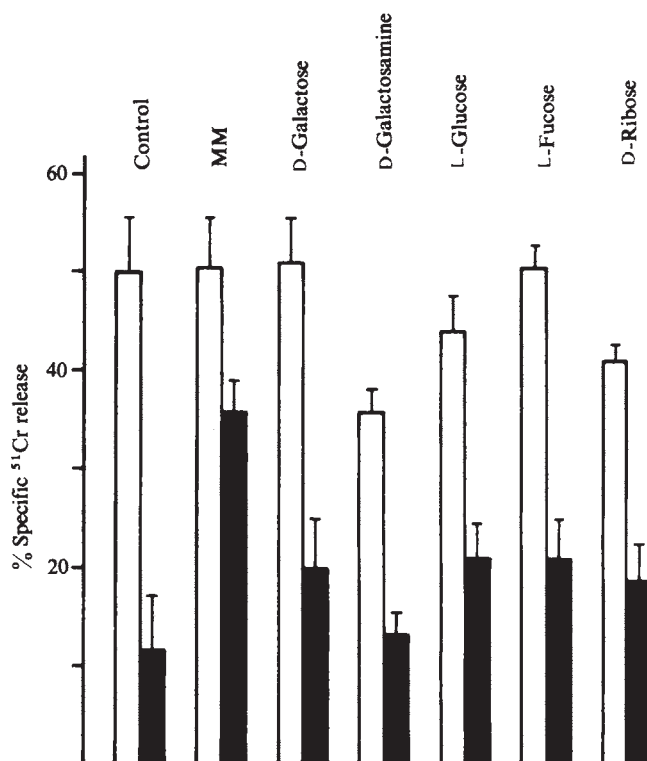


Fig. 4 Specificity of the carbohydrate effect on suppressor-cell functions. For suppressor and responder culture conditions, see Fig. 1 legend. The control shows the activity of responder cells incubated in absence (open columns) or presence (solid columns) of suppressor cells. In the other experimental groups different carbohydrates were added at 10^{-3} M concentration during the induction phase to the suppressed and non-suppressed cell cultures.

carbohydrates not toxic to effector-cell generation may not be sufficient to inhibit measurably suppression.

No definite function has been ascribed to oligosaccharides although their role in cell recognition has been suggested. Our observations on the counteraction of suppressor-cell activity suggest that carbohydrates play a part in T-cell regulation.

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Is erythropoietin the only factor which regulates late erythroid differentiation?

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Haematopoiesis is a useful model system for studying differentiation and the regulation of precursor cell populations intermediate between the multipotential stem cell and terminally differentiated end cells. For many years, erythropoietin (Epo) was recognized as the hormone which controls red cell production *in vivo*¹. Although other substances are now known to be required during the initial stages of erythropoiesis^{2,3}, late erythroid differentiation is regarded as strictly Epo-dependent⁴. This concept is supported by the recent demonstration that the addition of Epo alone to serum-free bone marrow cell cultures is sufficient to stimulate the CFU-E (colony-forming unit-erythroid—a late erythroid precursor cell approximating to a pro-erythroblast) to complete differentiation into mature erythrocytes.⁵ However, the data reported here indicate that mature erythroid colonies (indistinguishable from those formed by CFU-E+Epo) are formed when adult bone marrow cells are grown for 2 d in methyl cellulose cultures containing spleen cell-conditioned medium (SCM) but no added Epo. SCM is a rich source of growth factors^{6,7} and initial observations⁸ suggested that its 'Epo-like' activity could be attributed to: (1) Epo, or (2) a factor which enhances the activity of small amounts of Epo in the culture medium, or (3) a factor(s) distinct from Epo which is also capable of stimulating late erythroid differentiation. The experiments reported here, which include a partial characterization of the 'Epo-like' activity, support the latter interpretation.

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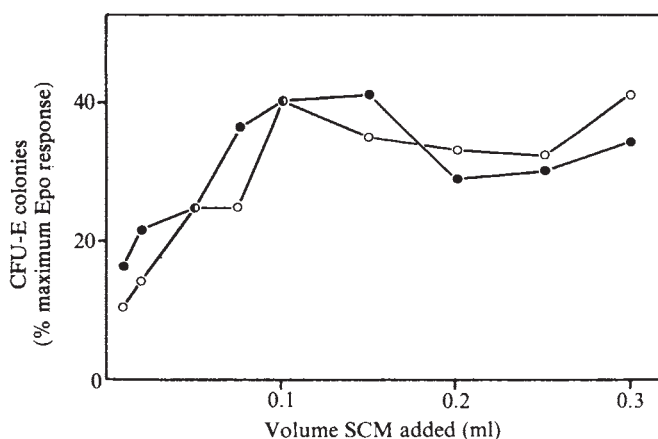


Fig. 1 Formation of erythroid colonies from adult mouse bone marrow cells grown for 2 d *in vitro* in the presence of different volumes of SCM. The number of CFU-E colonies is expressed as a percentage of the maximum response to Epo (0.15 units per ml culture; 307 ± 31 colonies per 10^5 cells, mean $\pm$ s.e.m. of 19 observations) in the same experiment. Epo was prepared from the urine of an aplastic anaemia patient according to Iscove *et al.*^{5,12}. Either 20% (●) or 0% (○) fetal calf serum was added to the culture medium. Bone-marrow cell cultures contained 0.8% methyl cellulose, fetal calf serum (0% or 20%), $32 \mu\text{g ml}^{-1}$ lecithin, $18 \mu\text{g ml}^{-1}$ cholesterol, 3.6×10^{-6} M human transferrin, 1% deionized BSA and 8×10^4 cells ml^{-1} . They were evaluated as described by Iscove *et al.*¹².

SCM was collected from mouse spleen cells which had been stimulated with allogeneic irradiated spleen cells⁹. Adult DBA/2J mouse bone marrow cells grown for 7–10 d *in vitro* in the presence of SCM produced granulocyte/macrophage, megakaryocyte, erythroid (bursts) and mixed colonies. Similar results have been described previously using this type of SCM or that obtained from pokeweed mitogen (PMW)- or concanavalin A (Con A)-stimulated spleen cells^{10,11}. The novel finding in the present study was that after 2 days *in vitro*, in the presence of SCM but no exogenous Epo, adult bone marrow cells produced colonies of 8–60 haemoglobinized (benzidine-positive) erythroid cells which were indistinguishable from those stimulated by Epo. Experiments designed to characterize this response in more detail showed that the number of erythroid colonies (cultured in the presence of 20% serum) increased in direct proportion to the amount of SCM added to the culture and reached a plateau value that was maintained at high SCM doses (Fig. 1). At a dose of SCM which maximally stimulated colony formation there was a linear relationship between the number of bone marrow cells plated ($0.25\text{--}4.0 \times 10^5$ cells per plate) and the number of erythroid colonies (correlation coefficient, $r = 0.98$). This indicates that the response occurs at the level of the individual cell and that colony growth is not dependent on cooperation between cells in the culture.

Fetal liver cells are often used to assay growth factors because they are generally more responsive and contain a higher proportion of precursor cells than adult tissues. SCM had a similar 'Epo-like' effect on 12-day fetal liver cells in culture to that on adult bone marrow cells. SCM from Con A- and PWM-stimulated spleen cells (one sample of the latter was provided by Dr G. R. Johnson, Melbourne, Australia) were also examined and found to contain a low level of 'Epo-like' activity when tested on adult bone marrow cells (data not shown).

All seven SCM preparations tested were active, although the level of 'Epo-like' activity varied between batches; the maximum response to SCM was between 41% and 80% of the maximum response to Epo, and required 33–150 μl of SCM per ml culture medium. The activity did not seem to be derived from the serum used to prepare SCM, because batches of SCM containing 1, 2 or 20% (v/v) serum gave similar results and

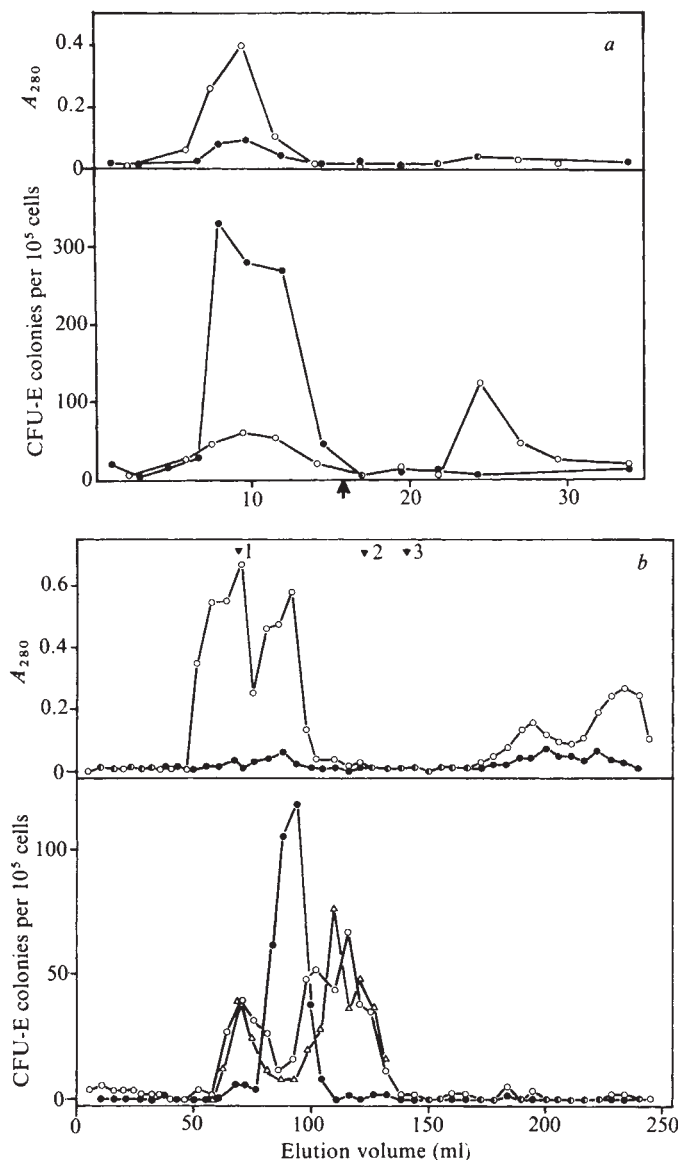


Fig. 2 Erythroid colony stimulating activity in fractions obtained by chromatography of Epo (●) and SCM (○, △) on (a) ConA-Sepharose and (b) Sephadex G-100 columns. The upper panel of (a) and (b) shows the A_{280} for each fraction: SCM contained 2% serum which accounts for the major peaks on these profiles. The lower panel shows the number of 2 d erythroid colonies formed in bone marrow cultures containing a 0.2 ml sample of each fraction. In all cases, the erythroid colonies were indistinguishable from those in control cultures containing Epo. a, ConA-Sepharose was equilibrated with running buffer (0.05 M sodium acetate, 0.5 M NaCl, 0.5 mM each of $MgCl_2$, $MnCl_2$ and $CaCl_2$, pH 6.5) and material bound to the column was eluted by addition of 0.1 M α -methylmannoside to the buffer (indicated by an arrow). Each fraction collected from the Con A column was dialysed against 0.16 M NaCl before assay. Similar results were obtained with other batches of SCM. b, Sephadex G-100 fine was equilibrated with 0.16 M NaCl, 4 mM HEPES, pH 7.4 containing 0.005% polyethylene glycol, which was also used to elute the samples. The increased A_{280} in the low-molecular weight region of the Sephadex G-100 profiles is due to phenol red which was present in the samples. Arrows 1, 2 and 3 indicate the positions of the MW standards, BSA (MW 69,000), α -chymotrypsin (MW 25,000) and ribonuclease (MW 13,700). The lower panel shows biological activity-elution profiles from two batches of SCM (○, △).

equivalent amounts of the same serum tested alone did not exhibit any 'Epo-like' activity on bone marrow cells.

Further experiments were carried out to test whether the 'Epo-like' activity in SCM was: (1) Epo released by the spleen cells, (2) a factor present in SCM which enhanced the activity of undetectable amounts of Epo in the serum used to prepare the bone marrow cultures or (3) another factor(s) distinct from Epo, which was also able to stimulate late erythroid differentiation. SCM gave a similar dose-response curve whether 20% (v/v) or no serum was included in the bone marrow culture medium (Fig. 1), and the number of colonies formed was not related to the amount of serum added (data not shown). These results suggest that SCM is not enhancing the activity of any Epo which might be present in the culture medium.

Does SCM contain Epo or another factor(s)? To answer this question a comparison was made between the physical properties of the 'Epo-like' activity in SCM and those of Epo. The biological activities of both Epo and SCM were unaffected by dialysis against 0.16 M NaCl (Amicon PM 10 membrane; 10,000 MW cutoff), or by heating at 56 °C for 30 min, but both were rapidly inactivated by boiling at 100 °C. However, the SCM activity could be distinguished from Epo by column chromatography. Two peaks of activity were detected when SCM was passed over a Con A-Sepharose column (Fig. 2a)—the first small peak was eluted immediately after the void volume in a similar position to Epo. The second larger peak was eluted with α -methyl mannoside and coincided with the granulocyte/macrophage stimulating activity (GM-CSA). Two regions of activity were also observed after chromatography on Sephadex G-100 (Fig. 2b), the first of which ran with the serum proteins and had a MW similar to a bovine serum albumin (BSA) standard. The second was more pronounced, had an apparent MW of 20,000–40,000 and overlapped with the GM-CSA. In contrast to the results from separation on Con A-Sepharose, no activity was detected where Epo elutes from G-100 (Fig. 2b). Thus, both affinity and gel filtration chromatography demonstrate that SCM contains an erythroid colony-stimulating activity which is physically distinct from Epo but has a similar biological activity. Protein sequencing studies are necessary to determine whether this factor contains any structural homologies to Epo.

These results indicate that SCM contains a factor(s) capable of stimulating late erythroid differentiation in the absence of exogenous Epo. Factors other than Epo have previously been shown to influence early erythropoiesis^{2,3} and in this respect it is interesting that Burgess *et al.*¹³ have recently isolated a glycoprotein (apparent MW 37,000 by Sephadex G-150 chromatography; 24,000 following neuraminidase treatment) which induces 7 d erythroid colonies in fetal liver cell cultures in the apparent absence of Epo. The authors concluded that this molecule was similar to burst-promoting activity^{2,3}; it possessed no Epo-like activity¹³ and earlier studies showed that only selected batches of serum supported the development of haemoglobinized 7 d erythroid colonies¹¹ (suggesting that additional factors were required for the complete maturation of erythroid precursor cells). Thus, the present study is the first to demonstrate that Epo may not be the sole regulator of the late stages of erythroid development. Further experiments are required to determine the physiological significance of this factor *in vivo* and its relationship with Epo and other growth factors in the regulation of haematopoiesis.

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Inhibition of histamine secretion from mast cells

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Histamine secretion from mast cells may be inhibited by elevated intracellular levels of cyclic AMP and by several anti-allergic drugs^{1–3}. These compounds are claimed to act directly on the calcium-gating mechanism activated by the anaphylactic reaction, preventing influx of Ca^{2+} from the external environment and so blocking exocytosis^{4–7}. To examine this hypothesis further, we have compared here the histamine secretion induced by immunoglobulin E-directed ligands in the presence and absence of added calcium and by the ionophore A23187. Exocytosis evoked by these former agents was originally considered to be almost totally dependent on extracellular calcium^{8,9} but recent studies have shown otherwise^{10–14}. In the absence of added cation, the agents act by mobilizing membrane-bound or intracellular stores of calcium^{10–12}. We show here that a variety of anti-allergic drugs are potent inhibitors in the conditions used, suggesting that alternative explanations for their action must be sought.

Rat peritoneal cells were recovered and histamine release determined as previously reported¹⁰. The effects of cromoglycate, theophylline and 8-bromo cyclic AMP on histamine release induced by antigen and concanavalin A (Con A) are shown in Table 1. The secretagogues produced similar releases of histamine in the presence and absence of added calcium^{11,12} and, as in our previous work with the basic inducers, peptide 401, compound 48/80 and polylysine^{11,15–17}, these drugs were comparably effective in both situations. The inhibitory effects of quercetin and doxantrazole were also essentially independent of added calcium.

Two important differences between ionophore- and antigen-induced histamine release must be considered. First, high concentrations of ionophore can induce much larger releases of histamine than the anaphylactic reaction. Second, the signal produced by antigen is transient and decays rapidly with time, whereas that evoked by the ionophore is much more persistent¹⁸.

The effects of cromoglycate and quercetin on the release induced by maximal and submaximal concentrations of ionophore are shown in Table 2. The compounds were effective inhibitors at the lower concentration of ionophore, which produced a release comparable with that evoked by antigen, but ineffective against the higher concentration. Theophylline, doxantrazole and 8-bromo cyclic AMP also inhibited histamine release induced by suboptimal amounts of ionophore¹⁹. These results are unlikely to reflect a simple interaction between the test drugs and the ionophore for several reasons. (1) The relative concentrations are inconsistent with such a model: cromoglycate (10 μM) inhibited significantly the release induced by the lower concentration of ionophore, whereas a 100-fold excess of drug was ineffective against a six-fold increase in the amount of

Table 1 Inhibition of histamine release induced by antigen and Con A in the presence and absence of extracellular calcium

	% Inhibition of release induced by:			
	Antigen		Con A	
	(+) Calcium	(-) Calcium	(+) Calcium	(-) Calcium
a, Cromoglycate (μM)				
200	—	—	55.1 $\pm$ 10.7	85.3 $\pm$ 6.8
100	61.7 $\pm$ 5.3	64.0 $\pm$ 10.0	51.5 $\pm$ 7.3	73.1 $\pm$ 6.4
50	62.9 $\pm$ 4.9	60.1 $\pm$ 10.1	46.8 $\pm$ 8.1	69.5 $\pm$ 7.3
25	57.5 $\pm$ 5.2	53.8 $\pm$ 10.0	42.6 $\pm$ 7.5	55.4 $\pm$ 7.3
10	43.2 $\pm$ 7.9	45.6 $\pm$ 10.2	31.5 $\pm$ 7.4	38.0 $\pm$ 6.6
5	22.8 $\pm$ 5.0	30.1 $\pm$ 13.3	25.0 $\pm$ 6.4	20.3 $\pm$ 3.2
1	6.3 $\pm$ 7.9	17.4 $\pm$ 6.9	8.9 $\pm$ 3.9	7.9 $\pm$ 1.2
b, Theophylline (mM)				
10	91.3 $\pm$ 6.7	95.4 $\pm$ 1.6	96.3 $\pm$ 1.2	100 $\pm$ 0.0
5	84.9 $\pm$ 6.2	77.2 $\pm$ 5.1	87.5 $\pm$ 3.5	85.4 $\pm$ 2.3
2.5	76.4 $\pm$ 8.1	57.7 $\pm$ 5.5	68.8 $\pm$ 8.7	57.7 $\pm$ 1.8
1	41.6 $\pm$ 8.0	30.8 $\pm$ 6.0	43.0 $\pm$ 7.7	31.4 $\pm$ 1.9
0.1	11.2 $\pm$ 4.8	-0.8 $\pm$ 2.6	2.6 $\pm$ 0.0	2.7 $\pm$ 1.1
c, 8-bromo cyclic AMP (mM)				
10	83.1 $\pm$ 4.1	96.0 $\pm$ 3.9	73.3 $\pm$ 3.2	80.4 $\pm$ 2.9
1	17.1 $\pm$ 8.3	23.0 $\pm$ 12.4	14.6 $\pm$ 1.8	-2.2 $\pm$ 9.8
0.1	4.6 $\pm$ 2.5	8.2 $\pm$ 8.2	1.9 $\pm$ 2.2	-7.8 $\pm$ 6.4

Rats were sensitized to the nematode *Nippostrongylus brasiliensis* and specific antigen was prepared as described previously¹¹. Cells (1 ml) were preincubated, as previously reported^{15,17}, in HEPES-Tyrod buffer¹⁰ containing calcium (1 mM) or EDTA (10⁻⁴ M) and then challenged with antigen (10 worm equivalents ml⁻¹) or Con A (20 μg ml⁻¹). Brief preincubation with EDTA enhances the secretory response in the absence of extracellular calcium, possibly by removing the cation from regulatory sites in the membrane and facilitating the release of more internal calcium stores^{11,12}. Secretion was allowed to proceed for a further 10 min, terminated by addition of ice-cold buffer (2 ml) and assessed as before¹⁰. Values are means $\pm$ s.e.m. Control releases induced by antigen were: a: (+) calcium 30.2 $\pm$ 5.3, n = 6, (-) calcium 32.4 $\pm$ 2.7, n = 6; b: (+) calcium 35.9 $\pm$ 5.0, n = 4, (-) calcium 34.6 $\pm$ 4.6, n = 4; and c: (+) calcium 29.5 $\pm$ 0.9, n = 3, (-) calcium 31.2 $\pm$ 3.2, n = 3. Control releases induced by Con A were: a: (+) calcium 39.0 $\pm$ 3.9, n = 4, (-) calcium 31.5 $\pm$ 2.6, n = 4; b: (+) calcium 38.5 $\pm$ 3.8, n = 4, (-) calcium 44.8 $\pm$ 0.8, n = 4; and c: (+) calcium 57.1 $\pm$ 1.7, n = 4, (-) calcium 30.3 $\pm$ 3.0, n = 3.

Table 2 Inhibition of histamine release induced by different concentrations of ionophore A23187

	% Inhibition of release induced by ionophore at a concentration of:	
	1.5 $\times$ 10 ⁻⁷ M	10 ⁻⁶ M
a, Cromoglycate (μM)		
1,000	63.5 $\pm$ 3.3	8.4 $\pm$ 4.5
500	55.5 $\pm$ 5.9	5.6 $\pm$ 2.8
250	50.2 $\pm$ 7.6	1.1 $\pm$ 1.4
100	42.4 $\pm$ 7.8	0.0 $\pm$ 0.9
50	33.5 $\pm$ 7.0	0.4 $\pm$ 1.0
25	32.8 $\pm$ 8.2	1.3 $\pm$ 0.1
10	25.1 $\pm$ 7.1	-1.5 $\pm$ 1.4
b, Quercetin (μM)		
25	87.7 $\pm$ 2.8	18.5 $\pm$ 6.6
10	75.0 $\pm$ 12.1	6.4 $\pm$ 0.9
5	58.4 $\pm$ 13.0	3.7 $\pm$ 1.0
1	36.1 $\pm$ 10.7	2.2 $\pm$ 0.6

Cells were preincubated with quercetin (5 min, 37 °C) and stimulated with the ionophore. Cromoglycate was added with the releaser. Secretion was assessed after a further 10 min of incubation. Values are means $\pm$ s.e.m. Control releases induced by ionophore at 1.5 $\times$ 10⁻⁷ M were: a, 36.0 $\pm$ 5.7, n = 11, and b, 14.7 $\pm$ 6.3, n = 5; and at 10⁻⁶ M were: a, 77.9 $\pm$ 1.9, n = 3; and b, 73.7 $\pm$ 2.2, n = 5.

Table 3 Effect of cromoglycate (200 μ M) on cells stimulated with ionophore A23187 (10^{-6} M) for different times

Time (s)	Control histamine release (% of total)	% Inhibition of release by cromoglycate
10	1.2 $\pm$ 0.4	—
20	32.3 $\pm$ 4.1	94.3 $\pm$ 1.5
30	41.7 $\pm$ 3.3	91.0 $\pm$ 2.4
40	44.5 $\pm$ 4.2	79.4 $\pm$ 3.5
60	48.8 $\pm$ 5.4	58.3 $\pm$ 4.2
90	49.2 $\pm$ 6.2	40.9 $\pm$ 4.4
120	52.1 $\pm$ 7.2	29.6 $\pm$ 10.7
300	69.3 $\pm$ 2.0	18.1 $\pm$ 2.7
600	75.3 $\pm$ 2.7	9.0 $\pm$ 5.9

Cells were stimulated with ionophore (with and without cromoglycate) for the period shown and the reaction terminated as in Table 1. Values are means $\pm$ s.e.m. for four experiments.

ionophore (Table 2). (2) The postulated interaction would have to apply to all the named compounds which represent diverse chemical types. (3) The drugs were active against maximal or supramaximal concentrations of ionophore if the cells were stimulated only for short periods of time (see below) or in the absence of extracellular calcium¹⁹. The concentrations required to effect inhibition in the latter conditions were generally appreciably less than in the presence of the cation and similar to those required to prevent antigen-induced secretion¹⁹. (4) This phenomenon is not confined to the ionophore and comparable effects (in which the extent of inhibition is dependent on the strength of the releasing stimulus) have been noted for various secretagogues^{11,15-17}.

To examine the time dependence of the inhibition, cells were stimulated with a maximal concentration of ionophore (with and without cromoglycate) and the reaction terminated after fixed periods. The control release of histamine increased progressively during the experiment but was potently inhibited by cromoglycate during the early stages of the reaction (Table 3). This effect is unrelated to the magnitude of the secretory response, as between 30 and 90 s of incubation there was only a slight increase in the amount of histamine released but a dramatic decline in the inhibitory effect of the drug. Thus, cromoglycate is a most potent inhibitor of exocytosis from cells stimulated with high concentrations of the ionophore for very short periods, conditions which mimic the transient signal produced by antigen. Identical results were obtained with doxantrazole and quercetin¹⁹.

The tested anti-allergic compounds therefore effectively prevent the release of histamine in conditions where the calcium-gating mechanism is circumvented (either by stimulating the cells in the absence of extracellular calcium or with the ionophore in conditions more closely resembling the anaphylactic reaction) and alternative explanations for their action should be sought. The compounds might modulate the calcium signal by activating membrane pumps to extrude the cation from the cytosol or promote sequestration of the ion into internal stores, as does cyclic AMP in other systems¹. Moreover, cyclic AMP and the anti-allergic compounds used here have been shown to prevent the accumulation of radiolabelled calcium by stimulated mast cells^{4,6}. This effect was attributed to a decreased influx of the ion (and taken as evidence for an inhibition of the gating mechanism) but is equally well explained by an increased efflux of the cation. Accumulation studies alone will not distinguish between these possibilities¹. Alternatively, the drugs might have effects not immediately related to calcium homeostasis and might promote disaggregation of microtubules²⁰, be involved in the phosphorylation of proteins in the plasma or perigranular membranes and so prevent the fusional changes involved in exocytosis^{14,21}, or have an as yet ill defined stabilizing effect on the mast cell membrane. Work to distinguish between these possibilities is now in progress.

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Persistent parainfluenza virus shedding during isolation at the South Pole

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Persistent parainfluenza virus shedding in healthy young adults occurred throughout the 8½-month winter isolation period at Amundsen-Scott South Pole Station during 1978. Two episodes of respiratory illness were observed after 10 and 29 weeks of complete social isolation. Throat swabs collected both routinely, and during each outbreak of respiratory illness, were directly inoculated into cell cultures. Parainfluenza virus types 1 and 3 were recovered from both symptomatic and asymptomatic subjects throughout the winter. No other viruses were obtained by these efforts. The presence of parainfluenza virus in these subjects long after the accepted incubation period for viral upper respiratory illness, and when the introduction of new virus to this community was impossible, suggests its persistence in man.

Upper respiratory viruses can be demonstrated just before the onset of coryzal symptoms and for a few days during convalescence. The incubation period of parainfluenza virus illness is short in both natural and experimental human infections and ranges from 2 to 8 days. In long-range epidemiological studies in children, parainfluenza viruses were recovered from daily throat swabs for a period of 3-10 days, with a median of 8 days¹. Similar brief virus shedding periods are observed following

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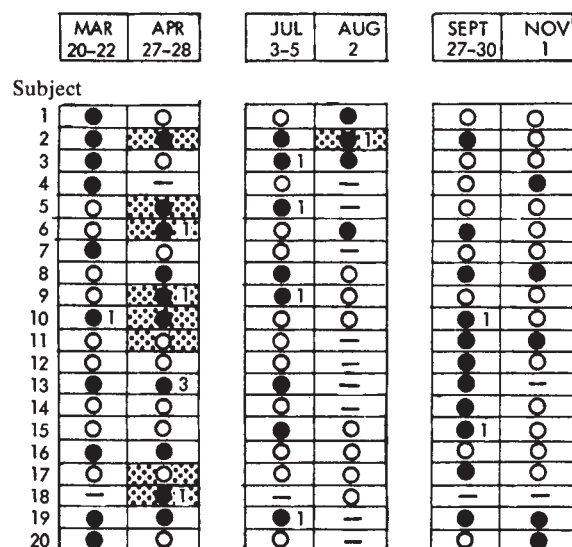


Fig. 1 Recovery of haemadsorbing viruses from throat swabs during the 1978 isolation period at the South Pole Station. Specimens were collected during the days shown for each month. The time interval between April and July and between August and September indicates that more than 30 days elapsed between specimens. ●, Virus recovered; ○, no virus recovered; —, no specimen obtained; [hatched box] indicates that the subject had an acute upper respiratory infection during this period; [boxed number] indicates that parainfluenza virus type 1 (or 3) was identified.

experimental infections in adults produced by nasal-oral inoculation of the parainfluenza virus². In long-term studies of respiratory virus infections within open communities, persistent parainfluenza virus shedding has not been observed³.

The American Antarctic Research Station at the geographic South Pole is totally isolated for 8½ months each year (mid-February to October) because the severe cold (−48 °C and below) prevents any aircraft operation. The summer period, extending from November to mid-February, is the time for station personnel changeover and re-supply. Each year since 1974, the South Pole physician has observed upper respiratory infections ('colds') among the Station personnel (~20 people) occurring after 2–6 months of total isolation, long after the usual short incubation period for these illnesses.

A limited virus laboratory was established at the South Pole Station, and operated during the austral winter of 1978 to attempt recovery and identification of the respiratory virus(es) responsible for these mid-isolation respiratory illnesses. Several continuous lines of mammalian tissue cells were maintained in culture by one of us (J.E.H.) throughout the year. These cell lines were human fetal tonsil, HeLa, Vero and Madin Darby canine kidney (MDCK). The last three lines were obtained as frozen suspensions from the American Type Culture Collection. The fetal tonsil line was isolated and tested by Schmidt *et al.*⁴ and obtained as a confluent monolayer.

Cell monolayers were grown as needed on the inner surface of 2.0-ml glass screw-capped vials. The monolayers were nourished using Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) and antibiotics. Before inoculation, the growth medium was removed and replaced with an appropriate maintenance medium. For HeLa and fetal tonsil cells, Eagle's MEM with 2% FCS and antibiotics was used, but to support growth of influenza viruses and parainfluenza viruses in MDCK and Vero cells, a serum-free medium consisting of Eagle medium 199 supplemented with extra glucose, vitamins and 0.02% trypsin was used⁵. All cell-culture manipulations were carried out under a fibreglass microbiological hood equipped with a UV light source. Removal of either growth or maintenance medium from the cell cultures established in 2.0-ml vials was accomplished with a fresh, sterile,

disposable, glass capillary tube connected to a closed-system vacuum source, and through suitable liquid traps, reducing the possibility of cross-contamination of cultures. Details of these miniature cell-culture methods were reported by Parkinson *et al.*⁶.

The winter isolation period in 1978 began on 15 February and continued until 1 November. Routine throat swabs and serum specimens were obtained (after informed consent) in March, July and October 1978 from the 20 wintering personnel, all healthy young men. Throat swabs were swirled in a virus transport medium containing penicillin and streptomycin, and the resulting suspensions were inoculated into the monolayer vials and incubated (35 °C) on a roller drum apparatus. Uninoculated control monolayers of all cell lines were set up simultaneously and processed identically with each group of specimens collected. Acute upper respiratory symptoms were noted in a few persons in two episodes beginning on 22 April and 2 August 1978. At those times, additional monolayers were inoculated and processed along with additional controls for the presence of virus by standard virological methods⁷. After inoculation, the original throat-swab specimens were frozen and retained at −70 °C.

During the winter, cultures showing either cytopathic effect or haemadsorption were passaged into fresh monolayers, incubated and stored with the original cultures at −70 °C. After station opening on 1 November 1978, the stored inoculated cell cultures and the original specimens were reinoculated into new cell monolayers from fresh cell suspensions transported to South Pole Station from the US.

Many viruses were recovered by these efforts. The haemadsorbing agents were best detected by the Vero cells, although weak haemadsorption by some isolates could be detected in fetal tonsil cells. None of the control Vero and fetal tonsil cells showed any haemadsorption. No haemadsorbing or other viruses were detected in the HeLa and MDCK cell lines. From the 20 winter-isolation volunteers, haemadsorbing virus was obtained from 48 of 105 throat-swab specimens, a 40% recovery rate. The viruses were identified as parainfluenza virus types 1 and 3 by haemadsorption-inhibition techniques, using known virus-specific antiserum (National Center for Disease Control). Not all virus isolates were typed as the immediately available supply of typing serum was exhausted by the unexpectedly large number of viruses recovered.

Table 1 Serological responses of subjects wintering at South Pole Station during 1978 to parainfluenza virus types 1 and 3 as determined by haemagglutination inhibition

	Subject no.	Sep 1977	Mar 1978	Jul 1978	Oct 1978	Nov 1978
Parainfluenza type 1	5	160	<u>160</u>	20	80	40
	6	40	<u>160</u>	80	80	80
	11	20	10	<u>10</u>		80
	16	<u>20</u>	<u>80</u>	80	80	80
	20	20	20	<u>40</u>	<u>160</u>	80
Parainfluenza type 3	5	320	<u>320</u>	40	80	160
	7	160	80	<u>160</u>	<u>40</u>	40
	8		<u>160</u>	40	<u>160</u>	160
	11	<u>160</u>	40	10		<u>160</u>
	15	40	<u>40</u>	<u>160</u>	80	80
	19		<u>80</u>	<u>320</u>	160	160

Serological responses (underlined) are defined as a fourfold or greater change in haemagglutination-inhibition titre between successive serum specimens.

Virus recoveries throughout the isolation period are recorded in Fig. 1. Some subjects (nos 7, 12, 14, 17 and 18) yielded virus in only one sample, but most subjects shed virus on two or more occasions. Two subjects (nos 13 and 19) shed virus into each specimen collected throughout the winter isolation period. The figure also indicates those subjects experiencing symptoms of upper respiratory illness during the isolation period. Most of the virus shedders were asymptomatic throughout the winter.

After the frozen serum specimens reached the home laboratories, haemagglutination-inhibition tests were performed by standard methods^{6,7} using commercial virus antigens (Microbiological Associates). Table 1 shows those subjects in whom four-fold changes of titre occurred. Subject numbers correspond to Fig. 1. Clearly, significant titre changes occurred in asymptomatic (nos 7, 8, 15, 16, 19 and 20) as well as symptomatic (nos 5, 6 and 11) individuals.

This remarkably high frequency of virus recovery indicates viral persistence and shedding throughout the isolation period in this small group of over-winter subjects. Such viral persistence in healthy subjects is contrary to present evidence that virus shedding in respiratory virus infections ends early in convalescence. However, in adult patients with chronic pulmonary disease, parainfluenza virus type 3 has been recovered on successive monthly occasions suggesting that this virus may persist in the abnormal respiratory tract⁸. The coryzal symptoms (colds) observed in the present group of subjects and in those of previous years occurred late in isolation, long after the presently accepted, short incubation period for parainfluenza virus infections. These late illnesses, occurring months into the total isolation period, also imply the continued presence of virus in an effective form which can be shed and cause disease. The South Pole environment is severely cold and dry. The mean ambient temperature is -47°C , with a range of -18°C . Only 1.5 cm of precipitation and 15 cm of new snow occur annually and the atmospheric water content (absolute humidity) averages 0.035 g m^{-3} . Whether this extremely cold, dry environment is significant in the observed parainfluenza virus persistence and shedding is unknown.

Allen *et al.*⁹ reported a correlation between changes in outdoor temperature and onset of respiratory illness which occurred after 17 weeks of total isolation among members of the 1969 British Antarctic Survey party at Adelaide Island on the Antarctic Peninsula. In another study¹⁰, subjects isolated at the British Antarctic Survey station, Stonington Island, for 5 months during 1971, showed an increased susceptibility to an experimental rhinovirus infection. An attenuated strain of virus was inoculated, but symptoms in subjects at Stonington Island were more severe than expected, with prolonged illness and virus shedding for a longer period (15 days) than observed in a control group inoculated with the same virus at the Common Cold Unit in Salisbury, England.

These earlier observations and our own reported here suggest that exposure of the upper respiratory tract to cold, dry air might impair the immune response of the respiratory mucosa. For example, this cooling and drying effect could lead to a reduced output of a secretory IgA and other anti-viral and anti-bacterial substances, resulting in either reactivation of an existing (persistent) viral infection or an increased susceptibility to reinfection.

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Monoclonal anti-Fc receptor IgG blocks antibody enhancement of viral replication in macrophages

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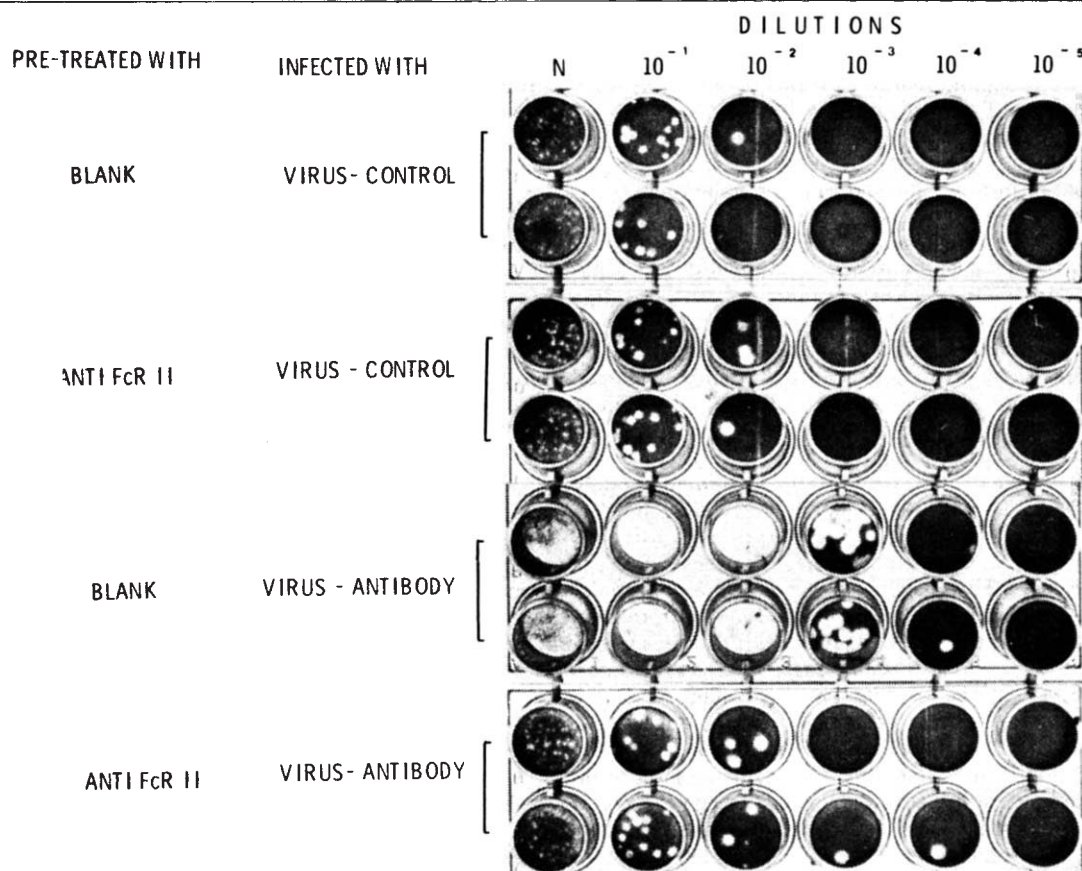
Flaviviruses, when complexed with antibody at subneutralizing concentrations, show enhanced replication in human and simian peripheral blood leukocytes (ref. 1, and J.S.M.P. and J.S.P., unpublished observations) and in P388 D1 and other macrophage cell lines². A comparable phenomenon has been demonstrated with alphaviruses and bunyaviruses in P388 D1 cells, (J.S.M.P. and J.S.P., unpublished observations) but cells lacking macrophage characteristics fail to show antibody-dependent enhancement (ADE) of viral replication. It has been suggested that the macrophage Fc receptor (FcR) provides an efficient route of entry of virus through the attachment of non-neutralized virus-antibody complexes and that for those viruses that escape destruction by the phagocyte, antibody results in a paradoxical increase in virus replication³. West Nile virus (WNV) replication in the P388 D1 macrophage cell line provides a reproducible model system for studying ADE of viral replication. Mouse macrophages have two FcRs—FcRI, which is trypsin-sensitive and binds IgG2a, and FcRII, which is trypsin-resistant and binds IgG2b and IgG1 complexes⁴. The FcR has been purified⁵ using rat anti-mouse FcR monoclonal antibody which blocks FcRII⁶. We show here that anti-FcR IgG and its Fab fragment block ADE of virus replication by anti-WNV monoclonal antibodies.

The cultivation of P388 D1 cells⁷ and preparation of WNV and rabbit anti-WNV serum have been previously described³, and mouse monoclonal antibodies were prepared (J.S.M.P. and J.S.P., unpublished) by standard techniques⁸. Ascites fluids of clones producing anti-WNV antibody of immunoglobulin subclass IgG1 (F7/101) and IgG2a (F6/16A) were produced. Immunoglobulin subclass was determined by Ouchterlony double diffusion using two independent sources of subclass-specific antisera (Miles; Meloy). Both antiviral antibodies react with WNV antigen to high titre in radioimmunoassay and mediate enhanced plaque formation to a dilution of 10^{-5} – 10^{-6} in P388 D1 cells. F7/101 neutralizes WNV poorly (50% plaque reduction at 1/10 dilution), whereas F6/16A neutralizes to a dilution of 1/1,280 (ref. 2). For the present experiments, both antibodies were used at subneutralizing concentrations (F7/101 at 1/1,000; F6/16A at 1/10,000). The production and characterization of the anti-mouse macrophage FcR monoclonal antibody and its Fab fragment have been described elsewhere⁶.

As the anti-WNV antibody-induced enhancement of WNV yields in P388 D1 cells is correlated with the number of infectious centres (J.S.M.P. and J.S.P., unpublished observations), we used an infectious centre assay (as described in Fig. 1 legend) to quantify ADE of viral replication.

Figure 1 shows that addition of anti-WNV antibody F7/101 resulted in a 189-fold enhancement in the number of infectious centres formed. This effect was blocked by pretreatment of P388

Fig. 1 Effect of anti-FcR antibody on ADE of WNV replication in P388 D1 cells. P388 D1 cells (2×10^6) were preincubated with anti-FcR antibody ($240 \mu\text{g ml}^{-1}$, 40 min, 4°C) and infected with WNV (multiplicity of infection 0.04) with or without antiviral antibody F7/101 in the continued presence of anti-FcR (2 h, 4°C). Cells were washed three times to remove unadsorbed virus and cell dilutions plated on a monolayer of pig kidney cells. An overlay of CM-cellulose was added 4 h later and plaques were stained after 4 days.



D1 cells with anti-FcR antibody, whereas virus replication in the absence of antiviral antibody was not affected. An irrelevant monoclonal antibody specific for mouse macrophages⁹ had no effect in this system.

We next compared the inhibitory effect of IgG and the Fab fragment of anti-FcR antibody and examined the effect of antiviral antibody subclass (Fig. 2). Anti-FcR antibody (IgG) almost completely blocked ADE of virus replication induced by either anti-WNV IgG1 or IgG2a antibodies (Fig. 2a), in

agreement with the ability of intact anti-FcR antibody to inhibit FcRI and FcRII in a macrophage rosetting assay⁶. In contrast, the Fab fragment of anti-FcR antibody showed less marked inhibition of ADE of virus replication, and no significant difference between the two subclasses (89% inhibition with F7/101 and 91% with F6/16A). The inhibition by anti-FcR Fab demonstrates that the inhibitory effect on ADE is due to the antibody combining site and not to the Fc domain. The reduced inhibitory activity of the monovalent Fab fragment may be due

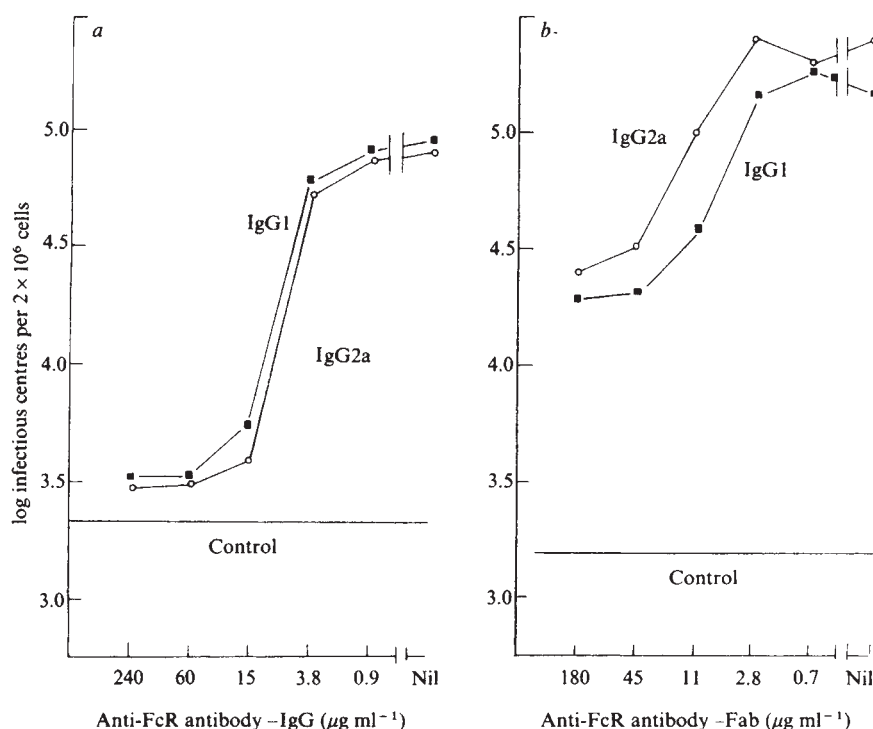


Fig. 2 Effect of the IgG (a) and Fab (b) fragments of anti-Fc receptor antibody on ADE of virus replication mediated by IgG1 and IgG2a anti-WNV antibodies. The procedures were as described in Fig. 1 legend.

Table 1 Comparison of the ability of anti-WNV IgG and F(ab')₂ to enhance replication of WNV

Rabbit anti-WNV	WNV antibody titre		Radioimmune binding assay	mg protein per ml
	Neutralization (50% plaque reduction)	Plaque enhancement		
IgG	1/40	1/2,000	1/3,125	0.86
F(ab') ₂	1/10	<1/3	1/625	0.46

Anti-WNV IgG was purified by DE52 (Whatman) chromatography and digested with pepsin. F(ab')₂ fragments were isolated on Sepharose S-200 (Pharmacia) and the absence of IgG confirmed by polyacrylamide gel electrophoresis. WNV neutralizing titre of antibody preparations was determined in pig kidney clone D cells¹². Plaque enhancement was titrated in P388 D1 cell monolayers. The titre is taken to be the highest dilution giving at least threefold increase in plaque counts over controls². For the radioimmunoassay, WNV antigen or mock antigen was coated on polyvinyl microtitre plates (Dynatech) and incubated with serial dilutions of antibody. Antibody binding was detected using ¹²⁵I-labelled conjugated rabbit F(ab')₂ anti-mouse F(ab')₂. The antibody titre was the highest dilution of anti-WNV giving at least twofold greater binding to WNV antigen than mock antigen¹³.

to the fact that binding to the FcR is reversible, whereas no measurable dissociation could be observed for the intact IgG⁵. The inability of Fab to discriminate between the IgG1 and IgG2 subclasses in ADE is surprising because previous studies showed that the same Fab fragment inhibited macrophage rosetting mediated by IgG1- and IgG2b-opsonized erythrocytes but was ineffective against IgG2a-mediated rosetting⁶. However, the binding of monomeric IgG2a to intact cells was inhibited by 15% by the anti-FcR Fab fragment and the binding of the purified FcR to IgG2a-coated beads could be completely inhibited⁵.

To confirm that the Fc fragment of the antiviral antibody was responsible for ADE, bivalent F(ab')₂ pepsin fragments of anti-WNV rabbit antibody were prepared. The F(ab')₂ antibody lost its ability to enhance viral replication but retained substantial neutralizing activity and ability to bind to virus antigens on radioimmune binding assay (Table 1).

Several lines of evidence suggest that ADE of virus replication is involved in the pathogenesis of viral disease. Virus replication *in vivo* (influenza; dengue) increases in animals previously infected with antigenically related viruses or following transfer of low levels of antiviral antibody^{10,11} and the dengue shock syndrome may be explained by this mechanism³. The present study shows that ADE of replication can be blocked by a monoclonal antibody specific for the Fc receptor. ADE demonstrated in human cultured peripheral blood monocytes can be abolished by removal of the Fc portion of the 'enhancing' antibody¹. However, the presence of the Fc receptor, though necessary, is not sufficient to show ADE. Resident mouse peritoneal macrophages and the lymphoblastoid cell line WEHI 231 which possess the same FcR determinant as P388 D1 (ref. 6) do not show ADE (J.S.M.P. and S.G., unpublished). Where ADE is observed, it is not clear whether the FcR facilitates virus entry directly or whether, by concentrating virus on the cell surface, it increases infection through an independent receptor for the virus itself.

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Murine DNA repair gene located on chromosome 4

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Xeroderma pigmentosum (XP) is an autosomal recessive human disease in which affected individuals are prone to develop skin cancers after exposure to sunlight. Cells from XP patients are defective in DNA repair activity and are sensitive to UV light. Most XP individuals have a defect in the excision repair pathway for UV damage. Genetic studies have indicated that excision-defective cells fall into seven complementation groups (A-G). An eighth group of XP is known to be defective in post-replication repair¹⁻³. DNA repair enzymes are ubiquitous and can function across species boundaries⁴⁻⁶. Primary mouse embryo fibroblasts have normal levels of DNA repair functions⁷. We report here that somatic cell hybrids between primary mouse cells and SV40-transformed XP group A cells can express wild-type levels of DNA repair function. These hybrid cells segregate murine chromosomes in culture. The proportion of cells in a given hybrid cell line which can perform unscheduled DNA synthesis (UDS) correlated well with the percentage of the population retaining murine chromosome 4. This study presents the first example of a direct quantitative comparison of specific gene activity and chromosomal content on a cellular basis.

Table 1 Statistical analysis of the relationship between retention of each murine chromosome and DNA repair activity in 21 hybrid lines

Mouse chromosome	Correlation coefficient	Slope	Intercept
1	0.422	0.446	34.1
2	0.445	0.451	34.3
3	0.486	0.587	22.4
4	0.940	0.992	2.01
5	0.599	0.625	28.9
6	0.554	0.775	32.6
7	0.485	0.789	34.4
8	0.611	0.538	28.6
9	0.564	0.620	28.9
10	0.438	0.617	30.6
11	0.527	0.768	28.9
12	0.391	0.390	26.5
13	0.612	0.805	28.1
14	0.558	0.721	33.0
15	0.740	0.774	16.8
16	0.575	0.623	25.9
17	0.658	0.614	25.0
18	0.401	0.413	22.0
19	0.364	0.435	27.7
X	0.451	0.676	34.4

The correlation coefficient *r* measures the degree of fit of the points on Fig. 2 (for chromosome 4), and similar plots for the other chromosomes, to the calculated least-square straight line. Slope indicates the number of murine chromosomes involved in conferring DNA repair ability on mouse-XP cell hybrids. Intercept represents residual DNA activity in the parental XP cells.

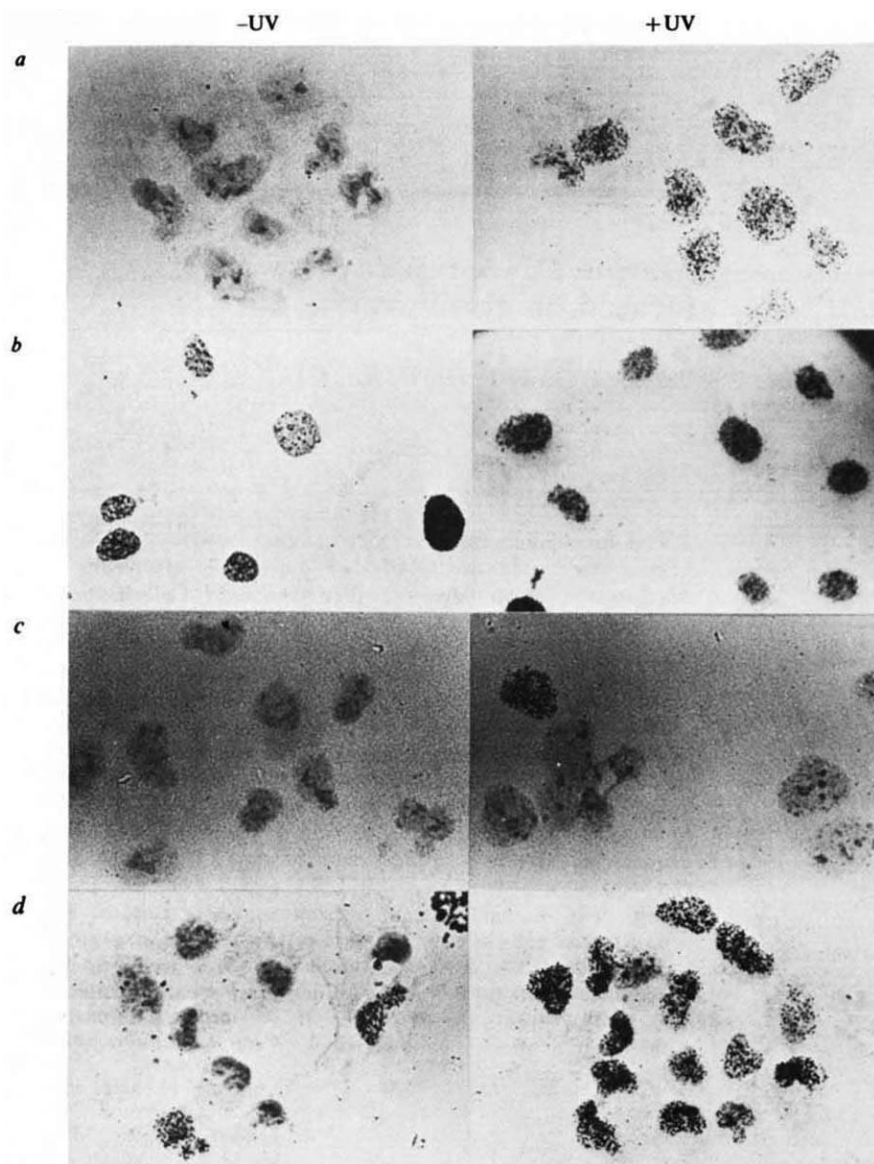


Fig. 1 Autoradiographs showing unscheduled DNA synthesis in parental and hybrid cell lines. Cells were UV-treated (30 J m^{-2}) and incubated in Dulbecco's modified Eagle medium + $10 \mu\text{Ci ml}^{-1}$ ^3H -thymidine (specific activity 20 Ci mmol^{-1}) + 2 mM hydroxyurea for 2.5 h after irradiation. After washing and fixation, slides were exposed to Kodak NTB-2 emulsion at 4°C for 7 days , developed then counterstained with Giemsa. Control cells were treated the same way, but were not UV-irradiated. *a*, SV40-transformed normal human cells, WI-18-VA-2 8-azaguanin resistant¹¹. *b*, C57BL/6J mouse embryo fibroblast (MEF). *c*, SV40-transformed XP group A cells, 1223. *d*, Mouse-human hybrid cells G_{31}B_2 produced by fusion of MEF and 1223. The dark spots in unirradiated mouse nuclei (*b*) are murine chromocentres.

Sixteen independent cell hybrids were generated by fusing C57BL/6J mouse spleen and SV40-transformed XP group A cells and selecting for ouabain 10^{-6} M -resistant colonies⁸ or by fusing microcells from mouse embryo fibroblasts and XP cells and selecting for UV (10 J m^{-2})-resistant colonies. The XP group A skin fibroblast (XP12BE) was obtained from the American Type Culture Collection. Cell fusion was promoted by 50% polyethylene glycol (PEG) 1540 (ref. 9) or concanavalin A (Con A) and PEG (A. Lalazar, personal communication). The hybrids were tested for DNA repair by assay for unscheduled DNA synthesis (UDS) after UV-irradiation¹⁰. As shown in Fig. 1, cell hybrid G_{31}B_2 which contains a full complement of human and mouse chromosomes expresses a normal level of DNA repair activity. This result provides another example of the lack of species specificity for DNA repair. However, we cannot determine which species contributes the active DNA repair enzyme. The functional enzyme may be the mouse form, or activation of the human form may occur in the presence of mouse regulating elements.

It has been shown that after several successive transfers of primary mouse embryo fibroblasts *in vitro*, the ability of these cells to perform excision repair decreases⁷. Note that in the stable hybrid G_{31}B_2 there is no apparent change in DNA repair capacity after 20 months in culture.

Most mouse-XP hybrids obtained segregated mouse chromosomes and contain an average of 60 human

chromosomes per cell. Chromosome analyses¹² and UDS assays were performed to determine whether there is an association between the segregation of a specific mouse chromosome and the expression of DNA repair function in mouse-XP cell hybrids. To confirm the karyotypic results, cell extracts from each hybrid were also assayed for the presence of 18 murine isozymes which have been mapped (by starch gel electrophoresis) in the mouse genome to 13 chromosomes¹³⁻¹⁵.

The DNA repair activity of cell hybrids was tested by UDS and the percentage of cells which could perform DNA repair calculated as follows:

$$\frac{\left(\frac{\% \text{ cells incorporating } ^3\text{H-thymidine after UV}}{\% \text{ cells incorporating } ^3\text{H-thymidine without UV}} \right) - \left(\frac{\% \text{ cells incorporating } ^3\text{H-thymidine after UV}}{\% \text{ cells incorporating } ^3\text{H-thymidine without UV}} \right)}{1 - \left(\frac{\% \text{ cells incorporating } ^3\text{H-thymidine after UV}}{\% \text{ cells incorporating } ^3\text{H-thymidine without UV}} \right)}$$

This represents:

$$\frac{\left(\frac{\% \text{ irradiated cells in S phase and in DNA repair}}{\% \text{ control cells in S phase}} \right) - \left(\frac{\% \text{ irradiated cells in S phase}}{\% \text{ control cells in S phase}} \right)}{1 - \left(\frac{\% \text{ irradiated cells in S phase}}{\% \text{ control cells in S phase}} \right)}$$

In the experimental conditions 50% of unirradiated SV40-transformed normal human fibroblasts (WI-18-VA-2) and SV40-XP A cells were found to be engaged in DNA replication. After UV irradiation (30 J m^{-2}) ~98% of the WI-18-VA-2 cells

showed DNA repair activity, whereas the value for SV40-XP A was only 2%.

In this study we analysed 21 hybrid lines (16 primary clones and 5 subclones), which cover the full complement of murine chromosomes except Y. To study the effect of each donor murine chromosome on the expression of DNA repair function in XP cell hybrids, the percentage of cells expressing DNA repair activity was plotted against the percentage of cells retaining a particular murine chromosome for the 21 hybrid lines. Figure 2 shows an example with mouse chromosome 4. For each of the 20 mouse chromosomes a least-square linear regression analysis of these two parameters yielded the results shown in Table 1.

The correlation coefficient r measures the degree of fit of the given points to the least-square straight line and the slope indicates the number of murine chromosomes which are involved in complementing the DNA repair defects in XP cells. The intercept represents the residual DNA repair activity in the parental XP cells which is about 2% (determined experimentally). The analysis shows that the hybrid cells which contain murine chromosome 4 have the highest correlation coefficient value (0.94) and the correct intercept value (2.01). Furthermore, in a Student's t test, for $r = 0.94$, $P < 0.001$. These data indicate that mouse chromosome 4 is responsible for the expression of DNA repair function in XP cell hybrids. The slope of 0.992 further suggests that murine chromosome 4 alone can complement the XP group A defect(s).

Another statistical analysis of expression of DNA repair function and karyotypic data for the hybrid line studies was carried out using a computer program¹⁶ and the results are summarized in Table 2. The positive correlation between the presence of an individual chromosome in a clone and that clone's ability to express DNA repair function was computed with three different measures of dependence. In all three cases, the expression of DNA repair activity was correlated with the presence of chromosome 4.

Table 3 lists the frequency of retention for mouse chromosome 4 and the percentage of cells which regain UDS for five subclones which were derived from four independent hybrids. These data further indicate that a change in the retention frequency of murine chromosome 4 is accompanied by a parallel change in the DNA repair ability in subclone lines.

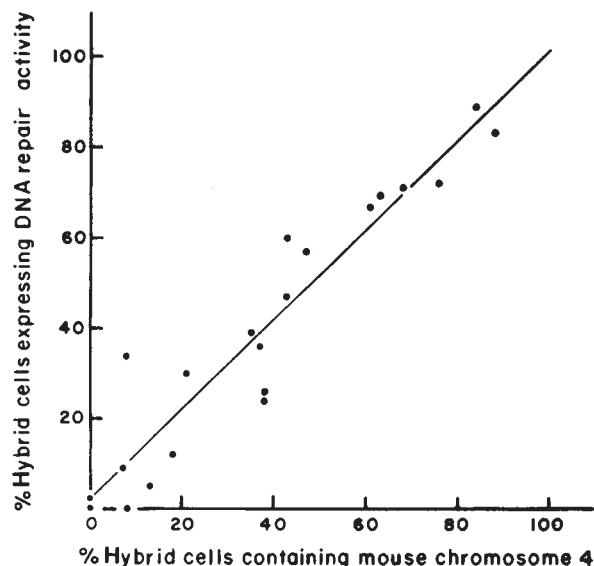


Fig. 2 The relationship between the presence of murine chromosome 4 and DNA repair activity in 21 murine-XP hybrid lines. Cells were treated as in Fig. 1 legend. Over 500 cells were counted for the incorporation of ^3H -thymidine for each hybrid. Cells were regarded as unlabelled if there were ten grains or less per nucleus.

Table 2 Assignment of murine gene responsible for restoring DNA repair activity in mouse-XP group A cell hybrids to chromosome 4

Chromosome	UDS/chromosome (no. of clones)				ϕ	χ^2	Overall OR
	++	+-	-+	--			
1	4	11	2	4	-0.07	0.09	2.69
2	4	11	1	5	0.11	0.24	2.20
3	11	4	3	3	0.22	1.05	7.38
4	14	1	0	6	0.89	16.80	10.56
5	6	9	1	5	0.22	1.05	3.45
6	3	12	1	5	0.04	0.03	1.90
7	3	12	0	6	0.26	1.40	2.19
8	7	8	0	6	0.45	4.20	5.03
9	6	9	2	4	0.06	0.08	2.92
10	6	9	1	5	0.22	1.05	3.50
11	6	9	0	6	0.40	3.36	3.87
12	11	4	4	2	0.07	0.09	7.60
13	5	10	0	6	0.35	2.62	2.97
14	4	11	0	6	0.31	1.98	2.44
15	11	4	2	4	0.37	2.91	7.26
16	8	7	1	5	0.33	2.35	6.38
17	7	8	2	4	0.12	0.31	4.58
18	11	4	3	3	0.22	1.05	6.91
19	10	5	5	1	-0.17	0.58	6.54
X	2	13	2	4	-0.23	1.11	1.37

Hybrid cells were considered positive for DNA repair function when over 20% of cells expressed UDS. For chromosomes (+) indicates presence of at least one copy in over 20% of the cells analysed. Statistical analysis was carried out using a computer program, ASSIGN, developed for the purpose of gene assignment with material generated by somatic cell hybridization¹⁶. To determine the gene assignment, three different statistical procedures were used: χ^2 , ϕ and OR. χ^2 was used as a method of dependence, rather than a test of significance, and was calculated in the ordinary way. The ϕ coefficient, which is insensitive to sample size is the ratio of the observed χ^2 to the perfect χ^2 . It is calculated by dividing the square root of the regular χ^2 by the square root of the sample size. When there exists a perfect association between a marker and a chromosome, the value of ϕ is +1. Zero indicates no association and -1 a negative association. The OR statistic was designed for determining gene assignments. It is an inverse weighted scoring system, independent of χ^2 , which is calculated by assigning each clone a score of 100 and dividing that value evenly among all chromosomes in concordance with the tested marker. The clones are scored, the scores added for each chromosome, and then divided by the number of clones. The chromosome with the highest number is considered a candidate for assignment of the gene under study. Generally an OR score of 7 or above is considered evidence for a positive assignment.

Table 3 The concordant segregation of chromosome 4 and the expression of DNA repair function in subclones

Hybrid line	Frequency of chromosome 4	% Cells with UDS
1. S $\times$ O ₂	61	67
S $\times$ O ₂ - 1	37	36
S $\times$ O ₂ - 50	76	72
2. S $\times$ A ₂	88	83
S $\times$ A ₂ - 1	21	30
3. S $\times$ R5	35	39
S $\times$ R5 - 1	43	47
4. G ₃₁ B ₂	84	89
G ₃₁ B ₂ - 1	43	60

Therefore we conclude that chromosome 4 contains a gene which corrects the defective DNA repair function in XP A cells. As it is not known if the XP A defect involves a structural gene change or an altered regulatory element, the gene product of chromosome 4 responsible for restoring DNA repair function remains undefined. Extending similar studies to other XP groups should help in elucidating the arrangement of murine DNA repair genes and mechanisms of mammalian DNA repair. The repair pathway for DNA damage in cells is complex, involving many different enzymatic activities; it is important to

determine where the genes for these enzymes reside in the genome and whether they are closely linked.

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Inducible repair of near-UV radiation lethal damage in *E. coli*

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We present here the initial characterization of a new repair system which is induced in actively growing cultures of *Escherichia coli* and which repairs a major fraction of lethal damage produced by near-UV (310–400 nm) light. This system is different from the error-prone 'SOS' repair system for DNA, known to be induced in *E. coli* by treatments which damage DNA and/or inhibit DNA replication, such as irradiation by far-UV (254 nm) light or X rays, thymine starvation or treatment with naladixic acid or mitomycin C^{1,2}. The SOS response requires induction of an 'X' protein³, the product of the *recA* gene^{4–6}, whereas the inducible repair system described here utilizes proteins distinct from the X protein.

The induction of a near-UV repair system different from SOS repair was initially inferred from the unusual survival pattern of *E. coli* K12 AB 2463 observed after irradiation with 366-nm monochromatic light. This strain carries the *recA*-13 allele, which renders it incapable of inducing the X protein or showing the physiological responses associated with SOS repair⁷. 'V'-shaped survival curves were obtained when exponential-phase cells were irradiated in conditions permitting protein synthesis during irradiation (Fig. 1). After the initial reduction of viability with fluences up to 70–100 kJ m⁻², further irradiation resulted in recovery of colony-forming ability; that is, cells, which would have been non-viable when plated after lower fluences, were viable when allowed to remain in suspension for additional irradiation before plating. Recovery is maximal after 200–250 kJ m⁻², the cells remaining resistant up to fluences of 500 kJ m⁻². Although the extent of killing and recovery can be modified by post-irradiation conditions, the V-shaped survival pattern remains, suggesting that the critical repair event(s) occurs rapidly, during irradiation and before plating. Addition

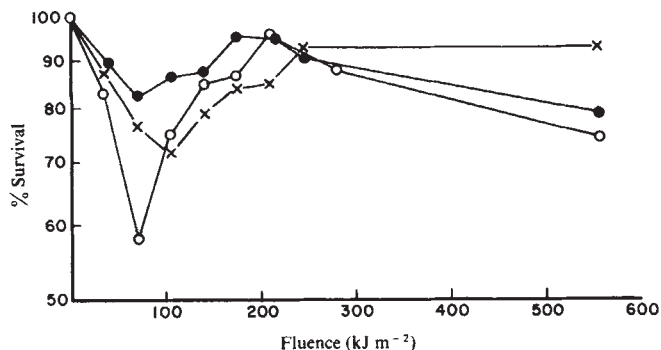


Fig. 1 Percentage survival of colony-forming ability of *E. coli* K12 AB 2463 (*recA*) as a function of 366-nm fluence in different post-irradiation conditions: plated on Luria agar (10 g l⁻¹ tryptone; 5 g l⁻¹ yeast extract; 5 g l⁻¹ NaCl; 15 g l⁻¹ agar) and incubated at 25 °C (x), plated on nutrient agar and incubated at 37 °C (O) or at 25 °C (●). Exponential-phase cultures were grown and irradiated (at 5–7.5 × 10⁷ cells ml⁻¹) at 37 °C in complete M-9 mineral salts medium¹² containing 0.4% glucose and supplemented with the auxotrophic requirements: arginine, histidine, threonine and leucine, each at 20 µg ml⁻¹, and thiamine at 5 µg ml⁻¹. The source of monochromatic 366-nm light was a Philips SP-500 high-pressure mercury arc lamp operated in conjunction with a Bausch and Lomb grating monochromator (model 38-86-45-49). A filter (Corning 0-52) was placed at the exit slit to screen out 340 nm and shorter-wavelength UV light. The fluence rate, measured with an Eppley thermopile, was 130 W m⁻².

of the protein synthesis inhibitor, chloramphenicol (CAM), at the start of irradiation, results in nearly exponential loss of viability, with only a slight low-fluence shoulder (Fig. 2). However, when CAM is added after the cells have received 200 kJ m⁻², there is a pronounced shoulder of about 40 kJ m⁻² (Fig. 2).

One interpretation of these results is that recovery of viability occurring in conditions that allow protein synthesis during irradiation is due to the induction of a repair system not present in unirradiated cells, or in cells receiving less than the inducing fluence of about 70 kJ m⁻². CAM added at the start of irradiation prevents induction of the repair system, whereas the shoulder observed when CAM is added after 200 kJ m⁻² reflects the time required for decay of induced repair protein(s) to

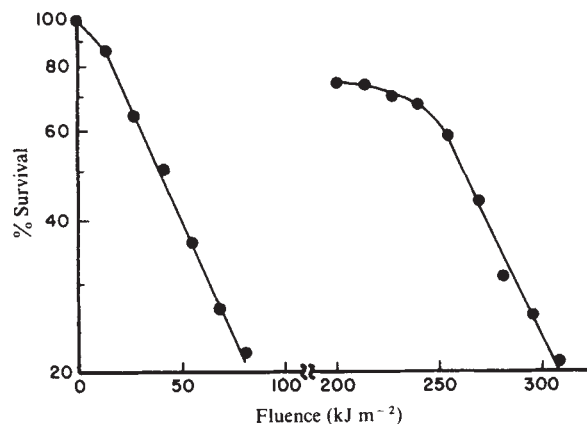
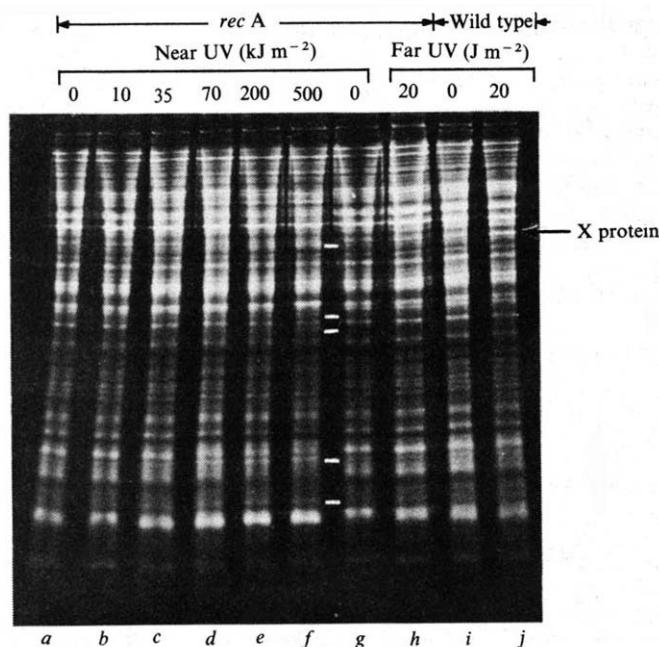


Fig. 2 The effect of inhibition of protein synthesis on colony-forming ability after near-UV (366 nm) irradiation. Cultures of *E. coli* K-12 AB 2463 were grown and irradiated as in Fig. 1 and plated on Luria agar at 25 °C. Chloramphenicol was added to the complete medium to a final concentration of 60 µg ml⁻¹ at the start of irradiation (left-hand curve) or after a fluence of 200 kJ m⁻² (right-hand curve). The fluence rate was 226 W m⁻². Complete medium irradiated with or without chloramphenicol had no phototoxic effect when incubated with unirradiated cells.

Fig. 3 Autoradiograph of SDS-polyacrylamide gel electrophoresis of total cellular protein of *E. coli* labelled with ^{35}S -methionine. The figure shows new protein bands appearing during near-UV (366 nm) irradiation of *recA* cells and comparison of the near-UV-inducible proteins with the X protein induced after far-UV (254 nm) irradiation of wild-type cells. Proteins made in unirradiated *E. coli* K12 AB2463 *recA* cells are shown in lanes *a* and *g*, and after increasing fluences of near-UV light in lanes *b-f*. The corresponding fluences are shown at the top of each lane. Proteins made in unirradiated wild-type *E. coli* K12 AB1157 cells are shown in lane *i* and in far-UV irradiated wild-type cells in lane *j* and *recA* in lane *h*. Bands of interest are highlighted by white lines in the gap between lanes *f* and *g*. The X protein in lane *j* is indicated by the arrow. Growth and near-UV irradiation conditions are as in Fig. 1. Cultures (0.5 ml) were pulse-labelled for 90 s in medium supplemented with $0.5\ \mu\text{g ml}^{-1}$ cold methionine and $50\ \mu\text{Ci ml}^{-1}$ of ^{35}S -methionine. Total cellular protein was solubilized and analysed by SDS-polyacrylamide gel electrophoresis using 14.5% gels according to the procedure of Studier¹³. Autoradiograms of dehydrated gels were made using Kodak X-Omat-R X-ray film. Pulse labelling of near-UV-irradiated cultures was done during continued irradiation; the far UV-irradiated culture was incubated for 20 min at 37 °C after irradiation before pulse labelling, to allow induction of the X protein.



physiologically insignificant levels. This period was estimated (from the ratio of the shoulder fluence to the fluence rate) to be 3.5 ± 0.5 min, and it remains constant over a threefold change in fluence rate. In contrast, the biological half life of SOS repair, as measured by the decay of error-prone repair activity, is 20–30 min (refs 8, 9).

This induced repair system must repair at least 90% of the damage produced at 366 nm, as may be seen by comparing the survival when the repair system is fully induced ($>200\ \text{kJ m}^{-2}$ fluence in Fig. 1) with that when its induction is prevented (left-hand curve in Fig. 2).

The interpretation of these survival data in terms of an inducible repair system is supported by our detection of new proteins synthesized during irradiation, whose appearance coincided with the recovery of colony-forming ability. Cultures of the *recA* strain were pulse-labelled with ^{35}S -methionine during irradiation with 366-nm light, and total cellular protein was analysed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. Lanes *a* and *g* of Fig. 3 show the pattern of pulse-labelled proteins from unirradiated *recA* cells, and lanes *b-f* show the proteins from cells that were pulse-labelled after increasing fluences of 366-nm light, from 10 to $500\ \text{kJ m}^{-2}$. Three new protein bands (which we call P_1 – P_3 in order of decreasing molecular weight, starting at the top of Fig. 3) appear at about $70\ \text{kJ m}^{-2}$ (lane *d*), and the bands corresponding to proteins P_4 and P_5 (indicated by the bottom two white lines in Fig. 3) show large dose-dependent increases in comparison with the unirradiated control. The relative intensity of the bands increases up to about $200\ \text{kJ m}^{-2}$ (lane *e*), at which time the recovery of viability is at a maximum (Fig. 1), with the pattern remaining essentially unchanged up to $500\ \text{kJ m}^{-2}$ (lane *f*).

Pulse-labelled proteins from unirradiated wild-type parental strain K12 AB 1157 are shown in lane *i* of Fig. 3. Lane *j* shows the proteins from wild-type cells that were pulse-labelled 20 min after irradiation with $20\ \text{J m}^{-2}$ of 254-nm far-UV light (the arrow indicating the X protein), whereas the same treatment failed to induce the X protein in the *recA* strain (lane *h*). This is generally the case with strains carrying mutant *recA* alleles in a variety of inducing conditions^{7,10}. Proteins P_1 – P_5 , which are inducible in *recA* cells by near-UV light, can be distinguished

from the X protein by electrophoretic mobility, consistent with the idea of a *recA* gene-independent, inducible repair system.

To distinguish near-UV-inducible repair from SOS repair, the above experiments were carried out in a *recA* strain, which lacks SOS repair. Although the wild-type parental strain did not show V-shaped survival curves, the following results indicate that the near-UV-inducible repair pathway also makes a major contribution to survival of the wild-type strain after near-UV irradiation: the extensive shoulder characteristic of near-UV survival curves is greatly reduced in the presence of CAM; the fluence at 366 nm required to reduce survival of strain AB 1157 to 10% of the unirradiated controls is $3,500\ \text{kJ m}^{-2}$ without CAM and $240\ \text{kJ m}^{-2}$ when CAM is added to exponential cultures at the start of irradiation. Proteins P_1 – P_5 are also induced in the wild-type strain by near-UV irradiation, as well as in the *recA* strain.

The maximal solar UV-A (320–400 nm) irradiance is about $5\ \text{mW cm}^{-2}$ at sea level¹¹. In these conditions, a fluence of $200\ \text{kJ m}^{-2}$ is delivered in about 1 h of exposure. Thus, the threshold fluence for the induction of the repair system described here is within the range of physiological exposure to UV-A, and we expect that this type of repair may be an adaptation to environments exposed to near-UV solar radiation.

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Evidence for the inducibility of the *uvrB* operon

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A new tool for probing the regulation of non-essential gene products has recently been developed¹—this is a derivative of bacteriophage Mu into which the *Escherichia coli* genes for lactose metabolism have been fused (referred to here as Mu(lac, amp)). The DNA of this defective virus can insert randomly into the chromosome of a cell and mutants can be retrieved in which the β -galactosidase gene carried on the phage is under the control of the regulatory elements of the operon in which it now resides. We used this phage to generate DNA repair-deficient mutants, one of which was highly UV-sensitive due to insertion of the Mu(lac, amp) into the *uvrB* region of the chromosome. When exposed to UV light, this strain produces β -galactosidase at a much greater rate than unexposed cells. This suggests that the *uvrB* operon is inducible. These results were initially rather surprising. Although various components of the enzyme systems involved in Weigle-reactivation^{2,3}, post-replication repair⁴⁻⁶ and repair of alkylation damage^{7,8} had been established as inducible, the products of the *uvrA*, *uvrB* and *uvrC* genes which collectively effect nucleotide excision repair⁹⁻¹¹, seemed to be firmly established as a constitutively synthesized repair system. This conclusion was drawn because the activity of these enzymes can be easily detected in uninduced cells⁹⁻¹¹; and mutations in the genes *recA* and *lexA*, which affect the regulation of most of the inducible repair systems¹², have relatively little effect on *uvrA*, *uvrB*-mediated excision repair¹³. Thus the *uvrA* and *uvrB* gene products seemed to be produced constitutively at maximal or near maximal rates. However, recent reports suggest a role for the *uvrA* and *uvrB* gene products in various inducible repair systems^{6,14-17}. Because these studies all relied on indirect measurements of induction, it could never be determined if the results reflected induction of other components of these systems or if the *uvrA* or *uvrB* gene products themselves were regulated. The insertion mutant described here allowed us to monitor expression of the *uvrB* operon directly and provided evidence for regulated synthesis of the *uvrB* gene product.

Our original UV-sensitive isolate, designated CON48, was a derivative of an HfrH strain, CA7092 (see Table 1). To characterize the source of UV-sensitivity in CON48, it was allowed to mate with a multiply-auxotrophic recipient, AB1157. After 30 min the mating pairs were disrupted mechanically and recombinants resistant to both ampicillin and streptomycin were selected. Of 110 Amp^r isolates tested, 74 were found to be Leu⁺, none were His⁺, and one was Arg⁺. Of a further 103 Amp^r colonies chosen, 97 were Gal⁺. Thus, CON48 seemed to carry the Mu(lac, amp) inserted into a chromosomal segment near the genes for galactose metabolism. The *uvrB* gene is also located in this region and mutants in this gene cause extreme UV-sensitivity^{13,21}; thus it seemed reasonable that the Mu(lac, amp) insertion was blocking expression of *uvrB*.

To test this hypothesis and to substantiate the map position of the Mu insertion, the transducing phage Pl_{cl} was grown on CON48. This lysate was used to infect JC3890, a strain deleted for the *uvrB* gene and several adjacent genes, including those for biotin biosynthesis^{20,21}. If Mu(lac, amp) was inserted in or near the *uvrB* gene of CON48, we anticipated that all Amp^r transductants would grow in the absence of biotin. This expectation was not fulfilled—most of the Amp^r transductants remained Bio⁻ while only a small fraction became Bio⁺. These Bio⁺ transductants were also Gal⁺ indicating that they had arisen by a

recombination event within the *gal-uvrB* region. One of these isolates was chosen and a temperature-resistant isolate, designated CON481R, which retained its Mu and ampicillin resistance but grew at 42 °C, was selected for further study. The selection of this revertant of the c^{ts} mutation carried on the Mu(lac, amp)¹, was performed so that the cells could be grown at temperatures which produce higher titres of Pl. When Pl lysates were prepared on this host and used to transduce JC3890, 100% of the 132 Amp^r transductants screened also became Bio⁺ and Gal⁺. These results suggest that CON48 contains more than one Mu(lac, amp) insertion whereas CON481R carries only one such insertion, that being in or very near the *uvrB* gene.

The *uvrB* region of CON481R was also transferred to AB1157. This produced ampicillin resistant, UV^s transductants. In this recipient only 5 of the 50 Amp^r UV^s transductants screened acquired a Gal⁺ phenotype. One such transductant was designated CON484 and used along with CON481R in further experiments. Because CON484 had originally been *uvrB*⁺, any UV-sensitivity it acquired must have resulted from the presence of the insertion mutation.

To establish that the Mu(lac, amp) insertion was in fact causing the cells to be *uvrB*⁻, two types of experiments were performed. First, the UV-sensitivity of CON484 was compared with that of JC3890 and AB1157. Figure 1 shows that the Mu(lac, amp) insertion produces the same UV-sensitivity as the *uvrB* deletion carried by JC3890. Similar results were obtained with CON481 (data not shown).

Host cell reactivation (HCR) results from the nucleotide excision repair of UV lesions mediated by the *uvrA* and *uvrB* gene products¹³. The ability of CON484 to carry out HCR was compared with that of AB1157 and JC3890. Figure 2 shows that CON484 was as deficient in this process as the *uvrB* deletion strain. These two experiments strongly argue that the Mu(lac, amp) insertion completely blocks *uvrB* expression either because it is inserted within *uvrB*, destroying the integrity of the gene sequence, or because it is inserted within the sequence of DNA coordinately regulated with the *uvrB* gene, but upstream from the gene, thereby totally blocking *uvrB* transcription.

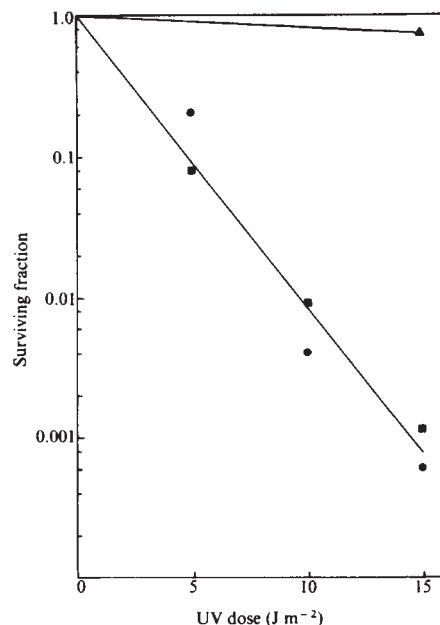


Fig. 1 Cultures (5 ml) of CON484, JC3890 and AB1157 were grown to saturation in L broth²². The cells were centrifuged out and resuspended in M9 salts²² at a density of 2×10^8 ml⁻¹. Samples (1.0 ml) of each culture were irradiated in watchglasses with varied levels of light from a Sylvania G30T8 germicidal lamp, maximum output at 254 nm. Appropriate dilutions were made and the cells plated on LB plates in 2.5 ml of soft agar. Plates were incubated at 30 °C for 36 h before colonies were scored ●, JC3890 (Δ uvrB); ▲, AB1157 (*uvrB*⁺); ■, CON484 (*uvrB*⁺:Mu(lac, amp)). PFU, plaque-forming units.

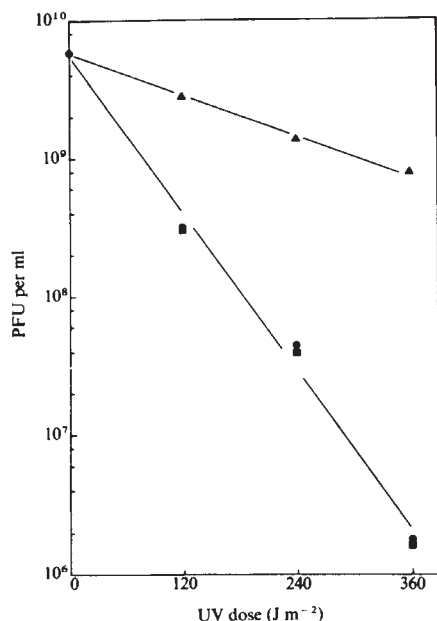


Fig. 2 Cultures of CON484, JC3890 and AB1157 were grown to saturation in T broth containing 0.2% maltose. A stock of λ c₈₅₇ in T broth²² was irradiated in a watchglass with varied levels of 254 nm light. The phage were then appropriately diluted in 0.01 M Tris-HCl (pH 7.5) containing 0.01 M MgSO₄ and added to 0.1-ml cultures of one of the three strains to be tested. Plaques were scored after 16 h growth on T plates at 37 °C. ●, JC3890 (Δ *uvrB*); ▲, AB1157 (*uvrB*⁺); ■, CON484 (*uvrB*::Mu(lac, amp)).

To establish finally that the Mu(lac, amp) insertion was located within the *uvrB* operon, strains were constructed in which a plasmid, derived from pBR322 and carrying the chromosomal region which includes the *uvrB* gene²³, was placed in cells carrying the Mu(lac, amp) insertion. This plasmid restored UV resistance to the multiply inserted CON48R as well as strains having only the single *uvrB* insertion. A similar plasmid carrying the same chromosomal segment but with an inactive *uvrB* gene left the insertion strains UV sensitive. We consider this to be very strong evidence that the Mu(lac, amp) insertion is within the *uvrB* operon.

The insertion of Mu(lac, amp) within the *uvrB* operon creates a situation where β -galactosidase is produced in response to stimuli which normally would cause increased synthesis of *uvrB* protein. Figure 3 shows that this operon is highly stimulated by exposure to UV light. During the 3 h following a 10 J m⁻² exposure, β -galactosidase levels within the cell increase 20-fold (this represents a 5–6-fold increase in β -galactosidase synthesis relative to other proteins). This UV dose produced a near maximum extent of induction. Exposure to other DNA damaging agents, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), also induces the synthesis of this enzyme but to a lesser extent. Cells without the insertion produce no β -galactosidase and non-irradiated cells continue to produce basal levels of the enzyme throughout the experiment.

The basal level observed from experiment to experiment is rather variable, ranging from 40 to 110 units for strains CON481R and CON484. In contrast to these relatively low basal levels, the original isolate, CON48, has an uninduced level greater than 200 units. Despite this higher level of uninduced β -galactosidase synthesis, its induced level is only slightly higher than that of the strains shown in Fig. 3. This is again consistent with CON48 carrying multiple Mu(lac, amp) insertions—the one in or near *uvrB* is inducible, whereas the other insertion(s) produces 100–200 units of β -galactosidase constitutively.

During the course of this work, a report was published which identified five other gene functions that were induced by UV light²⁵. One of these inducible operons was the *uvrA* gene, but no insertion into the *uvrB* gene was reported. All the UV-

inducible genes isolated by Kenyon and Walker seem to comprise part of a regulon which is controlled by the *lexA* and *recA* gene products. In an attempt to determine if the *uvrB* gene also belongs to this regulon, *lexA* and *recA* strains carrying the *uvrB*::Mu(lac, amp) insertion were constructed. Both the *lexA*1 and *recA*1 mutations blocked the UV induction of β -galactosidase seen in *lexA*⁺, *recA*⁺ insertion strains. Thus, the *uvrB* gene is probably part of the *lexA*, *recA* regulon.

The biological impact of these results is substantial. If the *uvrA*, *uvrB*-mediated excision repair system is controlled by the *lexA*, *recA* regulatory system, and the amount of excision repair capacity increases significantly in response to DNA damage, this could explain the observations of increased survival of UV-ray irradiated phage in cells carrying the *tsl* mutation¹⁴. It may also be responsible for parts of Weigle-reactivation^{16,17}, and post-replication repair⁶. If this is the case it could provide a significant simplification to an otherwise bewilderingly complex picture of UV repair.

However, a note of caution must be added. We have presented here evidence consistent with the idea that the *uvrB* gene is

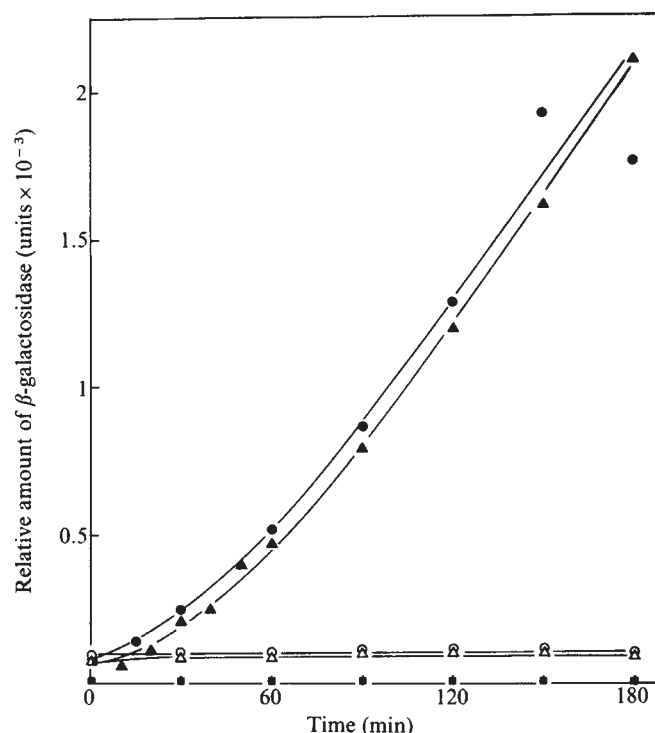


Fig. 3 Strains CON481R and CON484 were grown at 37 °C in M9 salts medium supplemented with 0.2% glucose, 2 μ g ml⁻¹ thiamine and 40 μ g ml⁻¹ Arg, Hist, Leu, Pro and Thr. When the cultures reached a cell density of $2-4 \times 10^8$ ml⁻¹, a 3-ml aliquot was irradiated with 10 J m⁻² of 254 nm light. A 2-ml sample of these cells was diluted in 8 ml of pre-warmed medium and returned at 37 °C to incubate. The non-irradiated control cells were diluted similarly. Samples (0.5 ml) were withdrawn at various times and added to 0.5 ml of Zcm buffer²⁴ on ice. The cell density was monitored at 600 nm. Because irradiated cells stopped dividing, the optical density of the initial point was used throughout the outgrowth as an indication of relative cell number. The optical density at 600 nm of non-irradiated cultures was monitored at each time point. After completion of sampling, toluene was added to make the cells permeable; the toluene was then evaporated at 37 °C and 0.2 ml of *o*-nitrophenyl- β -D-galactopyranoside (4 mg per ml in 0.1 M sodium phosphate buffer, pH 7.0) was added to each sample and the reaction allowed to proceed at 28 °C. The reaction was stopped by addition of 0.5 ml of 1.0 M Na₂CO₃. Relative amount of β -galactosidase is expressed as standard units which have been defined²² as $[(A_{420} - 1.75 A_{550}) / (vA_{600})] \times 1,000$. ○, Non-irradiated CON481R; ●, irradiated CON481R; △, non-irradiated CON484; ▲, irradiated CON484; ■, non-irradiated or irradiated AB1157.

Table 1 Genotypes and origins of strains used

Strain	Genotype	Source
CA7092	HfrH <i>thi1 ara Δ (proA, B-lac)</i> _{XIII}	Reznikoff*
AB1157	F ⁻ <i>thr1 leu6 proA2 his4 thi1 argE3 lacY1 galK2 ara14 xyl5 mtl1 tsx33 rpsL31 supE44</i>	Mount†
JC3890	Same as AB1157 plus <i>Δ (uvrB-pgi)</i> ₃₀₁	Kato‡
CON48	HfrH <i>thi1 ara Δ (proA, B-lac)</i> _{XIII} multiple inserts of Mu(<i>lac, amp</i>)cts	Amp ^r isolate of CA7092 infected with Mu(<i>lac, amp</i>)cts§
CON481	Same as AB1157 but carrying the <i>uvrB::Mu(lac, amp)</i> cts from CON48	Amp ^r bio ⁺ gal ⁺ transductant of JC3890 infected with Pl _{cl} grown on CON48§
CON481R	Same as AB1157 but carrying <i>uvrB::Mu(lac, amp)</i>	Temperature-resistant amp ^r Mu ^r isolate of CON481§
CON484	Same as CON481R	Amp ^r Gal ⁺ transductant of AB1157 infected with Pl _{cl} grown on CON481R§

* Ref. 18; † ref. 19; ‡ ref. 20; § this study.

part of an inducible regulon controlled by the *lexA* and *recA* gene products. This conclusion seems to make good biological sense. Unfortunately, it has been based on the analysis of a single insertion mutation. We cannot rule out the possibility that a genetic rearrangement could have occurred during insertion which fused the *uvrB* gene to a promoter other than the one normally used by *uvrB*. If this occurred, we have been analysing regulation at this promoter rather than the one normally used for synthesis of *uvrB* message. Although this is possible, it seems unlikely that a second extraneous promoter fortuitously fused to *uvrB* would be controlled by the *lexA* and *recA* gene products.

Another complication which could affect our conclusion arises from the fact that the Mu(*lac, amp*) insertion makes the cell UvrB⁻. If the *uvrB* gene is in any way autoregulatory, the absence of an active *uvrB* gene product could drastically alter the regulation of this operon. Experiments are being carried out to clarify this situation.

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Chromatin fine structure of active and repressed genes

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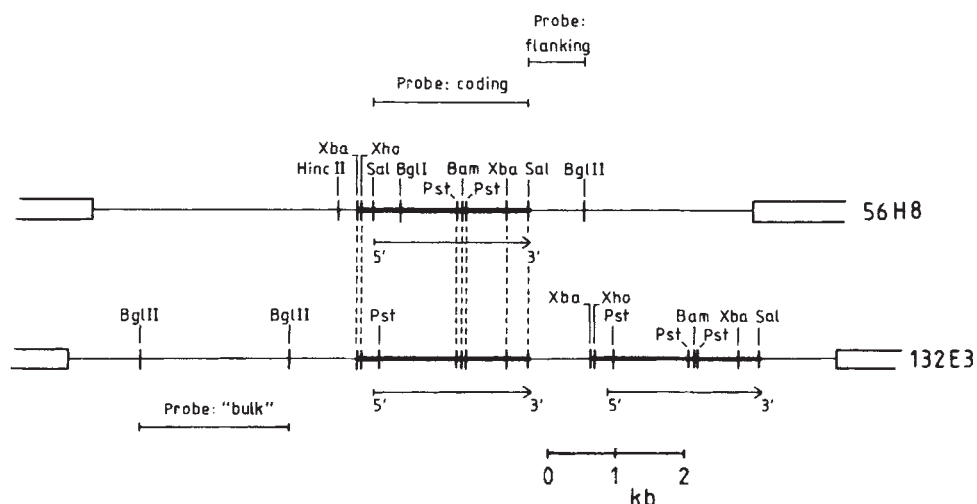
Study of the structural organization of chromatin during transcription and replication may reveal important aspects of these processes. At the lowest level of organization, chromatin consists of a repeating subunit, the nucleosome (for reviews see refs 1–3). Electron microscopy indicates that the nucleosomes are arranged helically^{4–6} or form discrete superbeads⁷, generating the familiar 250 Å–300-Å fibre⁸. It has been suggested that this fibre is further folded into loops containing up to several hundred nucleosomes^{9,10}. Despite extensive study, the significance and fate of these nucleosomes remain obscure. We have used here micrococcal nuclease digestion to compare the structures of actively transcribing and inert chromatin of the genes coding for the major heat-shock protein of *Drosophila melanogaster*. The repressed *hsp 70* genes were considerably more resistant to cleavage by micrococcal nuclease than their flanking regions and the bulk of chromatin. The active genes, previously shown to be more sensitive than the repressed genes^{11–13}, are also more susceptible to the nuclease than their 3'-flanking regions and bulk chromatin.

The heat-shock genes of *D. melanogaster* provide an excellent system for studying different aspects of gene structure and regulation (reviewed in ref. 14). These genes are induced by transferring *Drosophila* tissues or cell cultures from their normal temperature at 25 °C to elevated temperatures above 30 °C. The major protein synthesized at 37 °C—*hsp 70*—has a molecular weight of 70,000. Six copies of the gene coding for this protein exist in the haploid genome of Kc cells¹⁵; each has been extensively characterized by cloning and whole-genome Southern analysis¹⁵ (M.-E. Mirault, personal communication).

Nuclei of a *Drosophila* tissue-culture cell line were digested with micrococcal nuclease before or after heat shock. The DNA fragments obtained were separated according to size by agarose gel electrophoresis, denatured, transferred and bound covalently to diazobenzyloxymethyl (DBM)-paper¹⁶. By hybridization with radioactively labelled probes, the DNA sequences of interest were analysed selectively. Three different probes were used (Fig. 1)—the *Sal-Sal* fragment of 56H8 containing almost the entire coding region yet no flanking region, the *Sal-BglII* fragment of 56H8 adjacent to the 3' end of the coding region and the *BglII-BglII* fragment to the left of the coding region in the genomic clone 132E3. The last probe contains moderately repetitive sequences¹⁷ and was found to exhibit the same distribution of the nucleosomal repeat pattern (Fig. 2C) as bulk chromatin [revealed by staining the gel with ethidium bromide (Fig. 2A)] and hence is designated 'bulk'. Whenever the patterns revealed by these different probes are compared, they have been obtained by hybridizations with the same DBM-paper after release of the previous probe by denaturation.

The micrococcal nuclease pattern of the coding region (Fig. 2B) differs from that of bulk chromatin (Fig. 2A, C) in non-heat-shock conditions. The higher ratios of multimers to monomer in the lanes of the repressed gene (Fig. 2B) compared with the corresponding lanes representing bulk chromatin (control lanes in Fig. 2A, C) indicate that at least part of the coding region of the repressed gene is more resistant to micrococcal nuclease than bulk chromatin. This conclusion is corroborated by the observation that the average size of nick-translated DNA (bulk) obtained after nuclease digestion of

Fig. 1 Restriction map of *hsp 70* genes. Restriction maps of the *D. melanogaster* sequences in the hybrid plasmids 56H8 and 132E3 (ref. 26) have been published²⁷. Only a few selected restriction sites are indicated here; the distances have been slightly corrected by calibration with additional low-molecular-weight markers (*Hae*III digest of PM2 DNA²⁸). Sequences complementary to the *hsp 70* mRNA and the direction of transcription of these genes are indicated by arrows¹⁸. The protected domain of the repressed *hsp 70* genes is represented by a thick bar comprising the coding region and most of the *z_{nc}* region¹⁸. The probes for the coding (*Sal*-*Sal*) and the flanking region (*Sal*-*Bgl*II), and the probe revealing a pattern similar to bulk chromatin (*Bgl*II-*Bgl*II) are shown above and below the maps. The names of the restriction enzymes have been abbreviated as follows: *Bam*, *Bam*HI; *Pst*, *Pst*I; *Sal*, *Sal*I; *Xba*, *Xba*I; *Xho*, *Xho*I. kb, Kilobases.



non-heat-shocked nuclei is much smaller than that of the pattern revealed by hybridization of the same DNA with the coding region. (Compare lanes labelled 'nt' and 'c' of 6% acid-soluble DNA in Fig. 3. Considering that the label incorporated into nick-translated DNA is proportional to its mass whereas the label of the hybridized DNA is proportional to the number of DNA molecules, the observed difference is even greater than apparent from a mere comparison of the patterns of the autoradiogram.)

After mild digestions, the DNA of the repressed gene hybridizing to the coding region exhibits a relatively narrow size distribution with an average of about 2.5 kilobases (control lane of *a* in Fig. 2*B*). In a more extensive digestion, three sharp bands at 2.52, 2.34 and 2.16 $\pm$ 0.05 kilobases appear above a background (control lane of *b* in Fig. 2*B*). These bands are more clearly visible when the background is reduced by a shorter exposure (lane *b* at far right in Fig. 2*B*). Therefore, a region of 2.5 kilobases, larger than the coding region¹⁸ but which must contain at least part of the coding region, is more resistant to micrococcal nuclease digestion than its flanking regions when the gene is not expressed. To determine the right boundary of this protected domain, the *Sal*-*Bgl*II probe specific for the 3'-flanking region of one of the genes (Fig. 1) was used. As evident from Fig. 2*D*, the patterns resemble those of the bulk DNA (Fig. 2*A*, *C*) more closely than those of the coding region (Fig. 2*B*). The three bands between 2 and 2.5 kilobases are barely visible (control lane of *b* in Fig. 2*D*), and digestion is slightly inhibited in the repressed gene (ratio of monomer to oligomer DNA in lanes of control in Fig. 2*D*) compared with the bulk (Fig. 2*C*). This suggests that the protected domain and the *Sal*-*Bgl*II region overlap only slightly. In other words, the protected region of the repressed gene ends at a site close to the 3' end of the mRNA coding region¹⁸ (Fig. 1).

From the size of the protected domain of about 2.5 kilobases, we predict that its left end reaches beyond the 5' end of the mRNA coding region and maps close to the left boundary of the *z_{nc}* region¹⁸. A more precise localization of the 5' limit by direct mapping is complicated by the presence of repetitive sequences in this region.

No bands are ever observed between 2 and 2.5 kilobases in heat-shock conditions, even when digestion is very mild (<0.2% acid-soluble DNA in Fig. 3), so that the DNA containing the coding region exhibits about the same average size as the protected domain of the repressed gene. (The size of the bulk

DNA in such digests is on average 15 kilobases, as shown in the two lanes on the left in Fig. 3.)

During more extensive digestions, beyond 7% acid-soluble DNA, the protected domain of the inactive *hsp 70* genes is eventually degraded to smaller multiples of the nucleosomal repeat which are resolved significantly better than in bulk chromatin (control lane of *c* in Fig. 2*B*, and 11% acid-soluble DNA in Fig. 3). The largest fragments observed correspond to 12, 13 and 14 nucleosomes and co-migrate with the three bands of 2.16, 2.34 and 2.52 kilobases visible after milder digestions, whereas nucleosomal repeats larger than 10 are not resolved in bulk chromatin. It follows that the average spacing of nucleosomes is more uniform in the inactive gene than in bulk chromatin. Calibration with markers of known lengths shows that the nucleosomal repeat of the inactive gene is 180 ± 4 base pairs (Fig. 3).

The fragment sizes resulting from digestion of active genes are not distributed randomly, yet differ from those obtained after micrococcal nuclease digestion of bulk chromatin or of inactive genes. This results in bands ('hs' lanes of *b* and *c* in Figs 2*B* and 4) at positions between those of the familiar nucleosomal repeat. These bands are clearer in Fig. 4 where the DNA has been analysed in denaturing conditions. As the bands appear at roughly equivalent positions and intensities in denaturing (Fig. 4) and non-denaturing gels (Fig. 2*B*), the DNA fragments produced are largely double-stranded. These bands do not originate from the presence of preferential cleavage sites in the free DNA because digestion of free DNA of a mixture of 56H8 and 132E3 does not produce such bands after hybridization to the same *Sal*-*Sal* probe. Nor are they oligonucleosomes of which the DNA has been degraded from the ends because no such monosomes are observed. Moreover, in contrast to bulk chromatin, these structures do not show any metastable intermediates at any level of digestion but are readily degraded to very short pieces. This is demonstrated in Fig. 4 in which DNA fragments as short as 57 nucleotides have been transferred efficiently (shortest marker indicated on the left), yet very little hybridization is observed below 160 bases. The conditions of hybridization used permit detection of hybrids as short as 40 base pairs¹⁹.

The observed changes in chromatin structure depend completely on the level of transcription. After returning heat-shocked cells to the normal temperature (25°C), there is a gradual disappearance of the transcription-specific structures

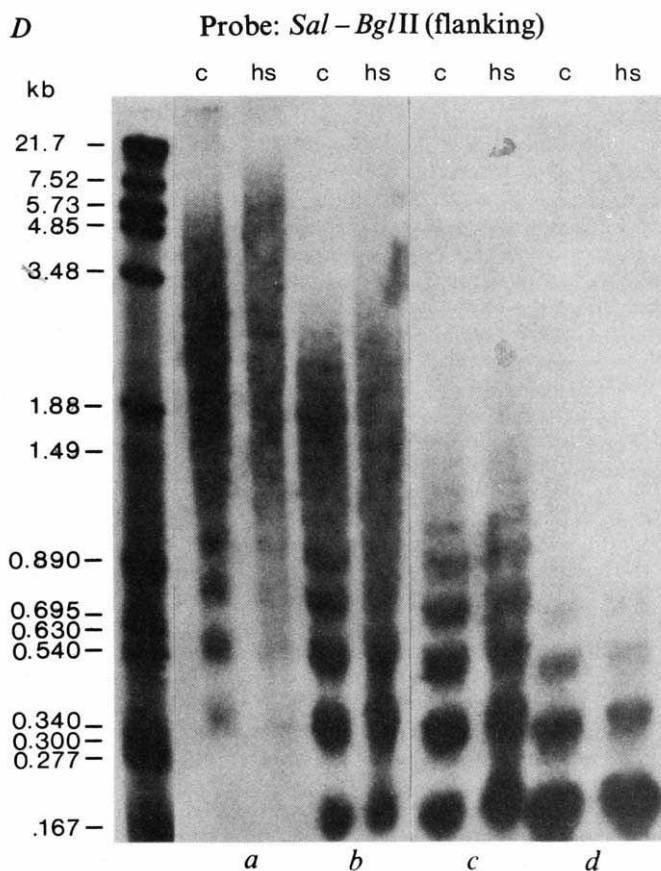
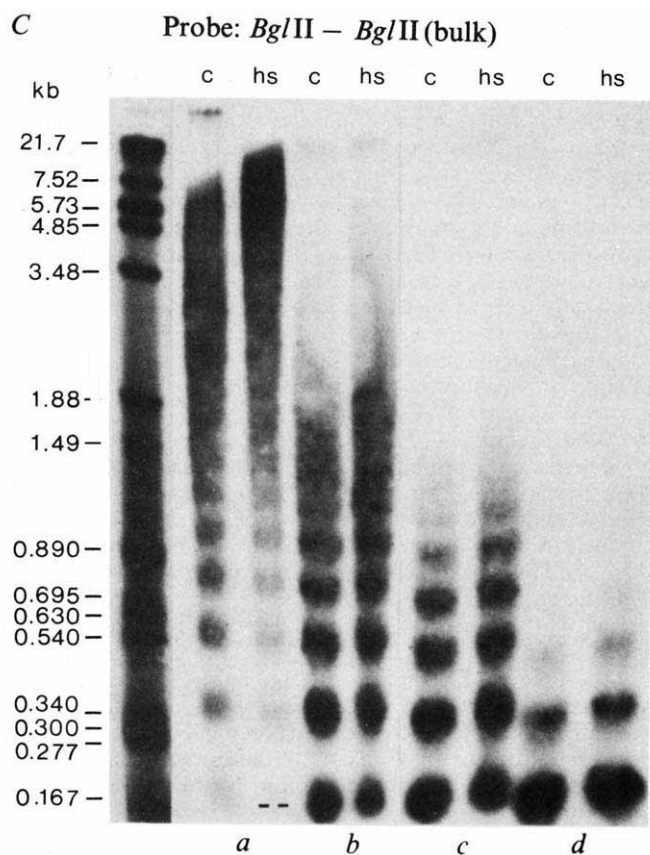
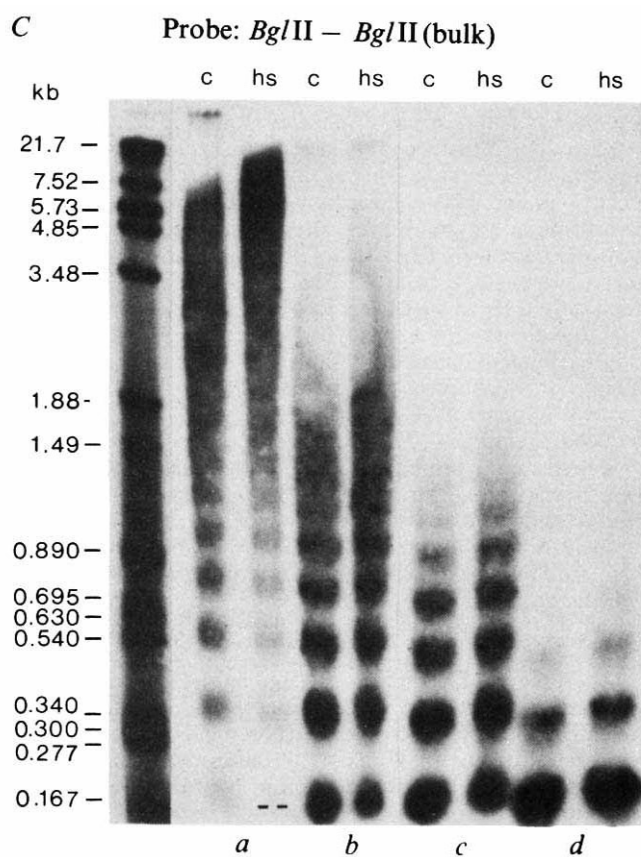
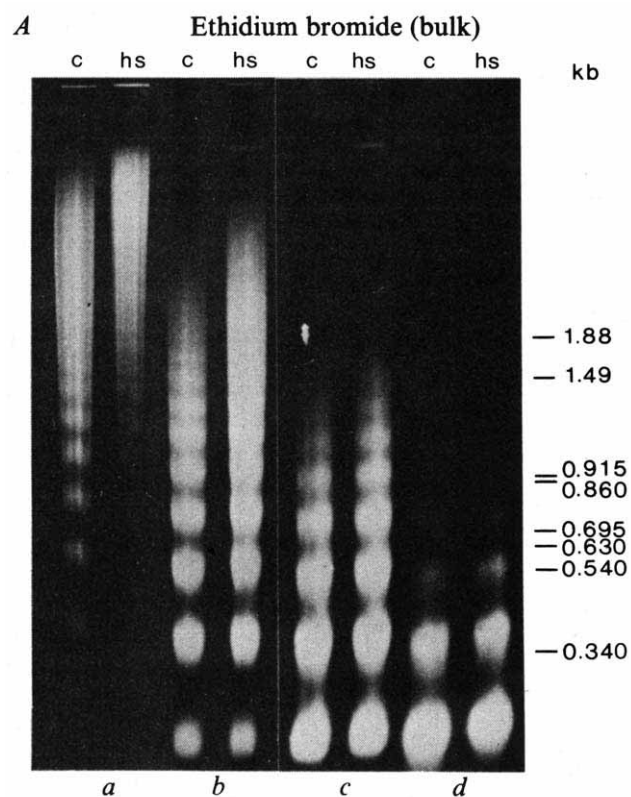


Fig. 2 Chromatin structure of *hsp 70* genes: comparison with structure of bulk and flanking regions. Digests of control (labelled 'c') and heat-shocked nuclei ('hs') were compared pairwise in bulk chromatin (A), in the coding region of the *hsp 70* gene (B), in a region containing a middle-repetitive sequence (C) and in the 3'-flanking region of the *hsp 70* gene (D). Four levels of digestion are shown in each panel corresponding to about 2.3% (a), 7% (b), 12% (c) and 18% (d) acid-soluble DNA. For preparation of nuclei, *Drosophila* Kc cells (adapted to growth in low serum) were grown in suspension at 25 °C in Echalier's medium²⁹ supplemented with 1% fetal calf serum (Gibco). Cells heat-shocked for 35 min at 36 °C, or control cells not subjected to heat shock, were collected in mid-log phase at a density of about 8×10^6 cells ml⁻¹. The cells were quickly chilled by pouring them on to half a volume of frozen balanced salt solution (BSS), and all subsequent steps were carried out on ice or at 4 °C. The cells were pelleted, washed twice in BSS and resuspended in Mg²⁺-buffer (0.33 M sucrose, 20 mM HEPES, pH 7.5, 80 mM NaCl, 1 mM MgCl₂) at 4×10^8 cells ml⁻¹. To lyse the cells, the cellular suspension was made 0.2% in Triton X-100, incubated for 15 min on ice and passed four times through a hypodermic needle (G-18). The nuclei were washed (1,400g for 10 min) two or three times in Mg²⁺-buffer and finally resuspended at a concentration of 1.2×10^9 nuclei ml⁻¹. For digestion, CaCl₂ to 1 mM was added to the nuclear suspension, and 0.5-ml aliquots were incubated for 2 min at 37 °C with 20 (a), 60 (b), 180 (c) and 360 units (d) of micrococcal nuclease (Worthington). The DNA was extracted and treated with a mixture of RNase A and T₁ RNase. Samples containing 5 µg (A) or 50 µg of DNA (B-D) were subjected to electrophoresis in two 1.7% agarose slab gels in 50 mM KNa₂PO₄, pH 6.5, 4 mM NaAc, 0.5 mM EDTA. One gel was stained with ethidium bromide (A), while the DNA from the other gel was transferred to DBM-paper and analysed by hybridization with various probes (Fig. 1); *Sal-Sal* of 56H8 (obtained as subclone *Sal-O*; B), *BglII-BglII* of 132E3 (C) and *Sal-BglII* of 56H8 (D). Before transfer, the DNA was denatured by washing the gel twice for 15 min in 0.5 M NaOH, neutralized in 0.5 M Na-phosphate, pH 5.5, for 15 min, and washed twice for 10 min in 11.5 mM Na-phosphate-citrate, pH 4.0, first at room temperature, then at 4 °C. For preparation of DBM-paper, a published procedure¹⁶ was modified to achieve an efficiency of DNA transfer of at least 40% over a size range of 40 bases to 21 kilobases. Using this modification, we can transfer at least twice as much DNA as was used in the experiments reported here before the binding capacity of the DBM-paper is reached¹². Hybridization with the nick-translated³⁰, ³²P-labelled probe was carried out in 0.02% each of Ficoll, polyvinylpyrrolidone and bovine albumin³¹, 5 × SSC, 1 mM EDTA, 0.1% SDS, 2 mg ml⁻¹ salmon sperm DNA. After hybridization, the paper was washed four times for 1 h at 65 °C in 2 × SSC, 0.5% SDS, 17 mM Na-phosphate, pH 7.5, 0.05% Na₂P₂O₇, 1 mM EDTA and finally briefly in water at room temperature. For autoradiography, preflashed³² Kodak XR-5 film was used with a Kyokko intensifying screen. All three autoradiograms have been obtained from one transfer of DNA to DBM-paper. After autoradiography, the hybridized probe was removed by four 30-min washes in 99% formamide at 80 °C, the DBM-paper was washed in water and the remaining ³²P-radioactivity (<3%) further reduced during 3-4 half lives to less than 0.3%. For calibration, the far-left lanes of each autoradiogram show *HaeIII* fragments of PM2 DNA²⁸ and *EcoRI* fragments of λ DNA³³ labelled at their 3' ends by 'Klenow' DNA polymerase I (Boehringer Mannheim). The lane on the far right in (B) represents a shorter exposure of the fourth lane from the left in (B). kb, kilobases.

(not shown). In some experiments (not shown), after micrococcal nuclease cleavage in the mRNA coding region of the active gene, we observed a smeared hybridization pattern initially which then gave way to a pattern reflecting the nucleosomal repeat. We could clearly correlate this smearing effect with a suboptimal heat-shock response. It thus seems crucial that transcription-specific structures be observed in heavily transcribed genes.

If the digestions after heat shock are relatively extensive ('hs' lanes of d in Figs 2B, 4), most of the hybridized DNA disappears, and only faint bands at the monomer and dimer positions of the inactive gene are visible. The residual monomer and dimer DNA can be explained if a few cells do not respond to heat shock or if a minor fraction of active genes contains small regions

of a structure similar to that of inactive genes. Inactivity of one or more of the six *hsp 70* genes in all cells after heat shock is unlikely because, as the milder digestions show, the quantitative differences between the active and repressed genes exceed a ratio of 6:1 (compare, for example, the region between 2 and 2.5 kilobases in the two lanes of b in Fig. 2B).

As even bulk chromatin was more sensitive to micrococcal nuclease than the repressed gene, it was of interest to compare the sensitivity of the active gene with that of bulk chromatin. Comparison of the corresponding lanes of digests obtained after heat shock in Fig. 2B with C shows a smaller average DNA size and a reduced amount of hybridization in the former. Thus the DNA of active genes is more sensitive than the DNA of bulk chromatin.

We also compared the initial rates of digestion of the active and repressed genes. The average DNA size of the active gene after digestion to less than 0.2% acid-solubility is about the same as that of the repressed gene after digestion to 6% acid-solubility for which 30 times more enzyme is required (Fig. 3). Thus the active gene is degraded 30 times more rapidly.

In addition, we conclude from a comparison of the amounts of hybridizable material in denaturing conditions in control and heat-shock DNA (for example ~1.9 kilobases in lanes of b or ~1 kilobase in lanes of c in Fig. 4) that both DNA strands of the active gene are more sensitive to micrococcal nuclease than those of the repressed gene.

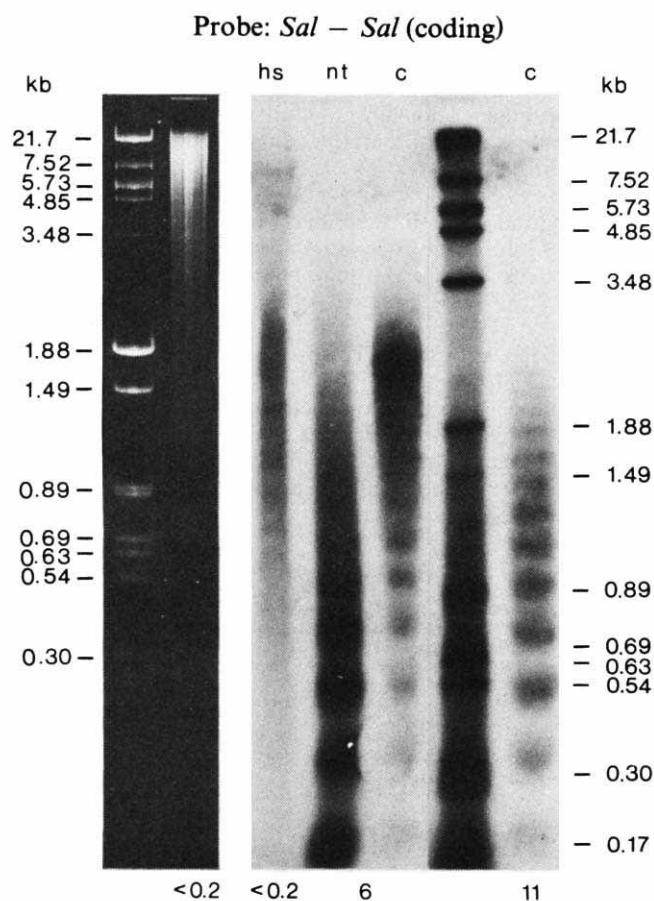


Fig. 3 Nucleosome spacing in repressed *hsp 70* genes and relative sensitivities of active and repressed *hsp 70* genes to micrococcal nuclease. On the left of the autoradiogram, the relative sensitivities to micrococcal nuclease of the active *hsp 70* gene ('hs'), of bulk chromatin ('nt') and of the repressed *hsp 70* gene ('c'); 6% acid-soluble DNA) are compared. At the far right, the uniform nucleosomal repeat of the repressed gene is shown ('c'; 11% acid-soluble DNA). Nuclei of heat-shocked ('hs') and control cells ('c') were prepared as in Fig. 2 except that the Mg²⁺-buffer was replaced by spermine-spermidine buffer³⁴ (0.33 M sucrose, 15 mM Tris-HCl, pH 7.5, 60 mM KCl, 15 mM NaCl, 0.5 mM spermidine, 0.15 mM spermine) and the concentration of Triton X-100 was raised to 0.4%. Digestion of heat-shocked nuclei (1.2×10^9 ml⁻¹) with 4 U ml⁻¹ of micrococcal nuclease and digestion of control nuclei with 120 or 360 U ml⁻¹ rendered <0.2%, 6% and 11% of the DNA acid-soluble, as indicated below the corresponding lanes. The DNA was extracted, and 50-µg samples were subjected to electrophoresis in 1.7% agarose gels, transferred to DBM-paper and hybridized to labelled *Sal-O* DNA as described in Fig. 2. The 'nt' lane shows nick-translated DNA of the digest generating 6% acid-soluble DNA. The second lane from the right contains the same markers as in Fig. 2. The two lanes on the left show the same marker DNA and DNA of the digest of less than 0.2% acid-solubility after staining with ethidium bromide.

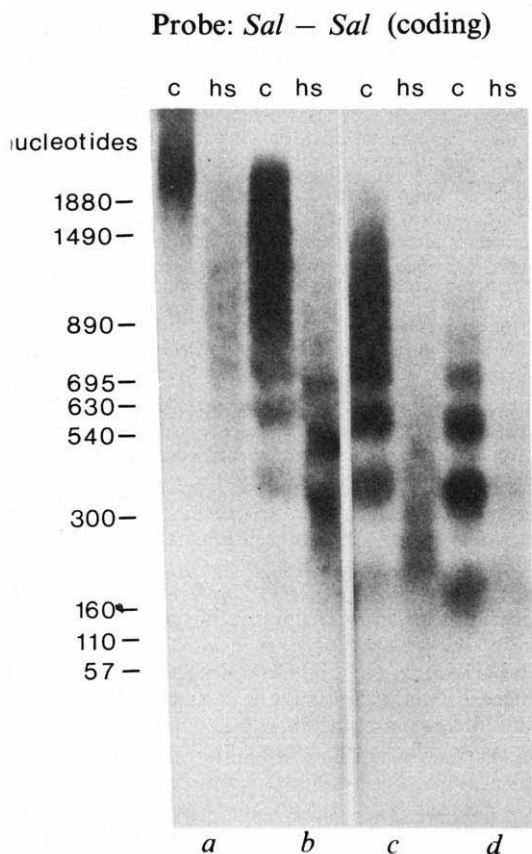


Fig. 4 Analysis of DNA fragments of the *hsp 70* gene in denaturing conditions. DNA samples (50 μ g) of the same digests of control ('c') and heat-shocked nuclei ('hs') shown in Fig. 2 were subjected to electrophoresis in an alkaline 1.7% agarose gel³⁵. After electrophoresis, the DNA was neutralized, transferred to DBM-paper, hybridized to *Sal*-O DNA and autoradiographed as described in Fig. 2 legend. On the left, the positions of *Hae*III fragments of PM2 DNA, used for calibration, are indicated.

As genes may be part of large loops of domains^{9,10,20,21}, it was interesting to examine whether the sensitive region of the active state extends beyond the coding region to include such larger domains. A comparison of the hybridization pattern of the 3'-flanking region (Fig. 2D) with that of the coding region (Fig. 2B) shows that the enhanced sensitivity does not extend much beyond the 3' end of at least one of the *hsp 70* genes (a similar study with the 5'-flanking region is complicated by the presence of repetitive sequences). The pattern obtained by hybridization to the 3'-flanking-region probe (Fig. 2D) is very similar to that of the bulk (Fig. 2A, C). Thus, for a first approximation, the region downstream from the right *Sal* site (Fig. 1) is organized similarly to bulk chromatin in heat-shock conditions. However, the patterns revealed by these two probes do differ slightly. First, the heat-shock lanes of b and c in Fig. 2D show more hybridization in the regions between monomer and dimer and between dimer and trimer DNA than the corresponding non-heat-shock controls or than the same lanes representing bulk chromatin (Fig. 2A, C). This suggests that the *Sal*-*Bgl*II probe overlapped slightly with the chromatin region containing the transcription-specific structure (Fig. 2B). Second, there is less hybridization after extensive digestion of the heat-shock-treated nuclei ('hs' lanes of d) in the flanking region (Fig. 2D) compared with bulk DNA (Fig. 2C) when normalized to the control. We infer that the sensitive portion of the gene ends close to the right-hand *Sal* site. As the 3' end of the mRNA coding region also maps close to this site¹⁶, the 3' end of the mRNA coding region and the right boundary of the nuclease-sensitive chromatin region must be very close to each other.

Altered sensitivity to nuclease of actively transcribed genes may result from several structural changes: (1) changes in the

higher structural orders, (2) changes in the structure of the nucleosomes, and (3) the absence of histones from transcribed DNA. Therefore, earlier suggestions made solely on the basis of DNase sensitivity that active genes contain structurally modified nucleosomes are invalid^{22,23}. More detailed information with respect to the structure of active genes may be gained by analysing the size distribution of the DNA fragments produced by the action of DNase, and this study has revealed transcription-specific structures not previously observed. Micrococcal nuclease was found to discriminate between a gene, regardless of its state, and the rest of the chromatin. The repressed genes were protected and the active genes were highly sensitive compared with bulk chromatin or the neighbouring non-transcribed region. The levels of sensitivity of the gene are reversible and depend on its transcriptional activity. Hence, models of gene repression need not be based solely on the interaction of regulatory proteins with the 5' end. Similarly, the association of bulk-type nucleosomes with the gene seems, in itself, insufficient to keep the gene in the inactive form. Some mechanism seems to exist which, on repression, alters the chromatin structure of the gene so as to reduce its accessibility (and that of a region of about 1 nucleosome at its 5' end) to the nuclease considerably below that of the flanking, non-transcribed DNA sequence.

The mechanism of protection of the repressed genes could be explained on at least two structural levels. Each linker region joining adjacent nucleosomes could be modified in such a way as to reduce its sensitivity to micrococcal nuclease. The known sequence of the *hsp 70* gene^{24,25} rules out the trivial explanation that all linker regions are less sensitive to micrococcal nuclease because of a high G + C content. In a more attractive model, all the linker regions of the entire domain become collectively resistant to the nuclease by some change in higher order of chromatin structure. The transition points between the protected domain of the repressed state and the flanking, bulk-like structure might then be sequences of regulatory significance. Specific nonhistone protein(s) interacting with these sequences may associate with each other to bring the two ends of this chromatin segment into tight proximity, producing a loop containing 14 nucleosomes. Such a loop may form a supercoiled, more compact and less accessible form of chromatin. In this model, activation of a gene would require at least two steps. First, the protected domain is unfolded, and only then does initiation of transcription start by the modification or removal of the nucleosomes.

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Theory agrees with experimental thermal denaturation of short DNA restriction fragments

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Experimental melting transitions of several natural DNAs of known nucleotide sequences have recently been obtained. The differential melting curves of these DNAs— Φ X174 DNA^{1–3}, fd DNA⁴ and SV40 DNA⁵—all show distinctive sets of peaks or fine structure. Theoretical melting curves calculated from the sequences and a few *a priori* parameters have not accurately predicted the experimental transitions^{2,6,7}. Although calculated fine structure resembled experimental curves in some cases, the characteristic features of a DNA's differential melting curve could not generally be produced. Azbel^{8,9} and Gabbarro-Arpa *et al.*⁵ have recently obtained good agreement between calculated and experimental curves using a different theoretical approach—only ground-state configurations of DNA were considered for temperatures inside the transition region. Their results suggest that the basic model of DNA melting, common to all theoretical approaches, is accurate. We have used here an exact theoretical approach to calculate melting curves of four DNA restriction fragments of 95–301 base pairs containing the lactose promoter region (Fig. 1). Theoretical curves agree very well with the experimental transitions published by Hardies *et al.*¹⁰ and obtained in this laboratory.

Figure 1 shows the four fragments relative to the *lac* promoter–operator region. The base sequence of this region was determined by Dickson *et al.*¹¹. Figure 2 shows the comparison between theory and experiment for the *lac* fragments in 0.1 M Na⁺. Theoretical curves predict the melting temperatures, T_m s, peak heights and widths at half height for the four fragments. Although short fragments do not have as much detail in their melting curves as long DNAs, the fine structure observed in Fig. 2 is predicted.

Theoretical curves were generated using the loop entropy model of the DNA helix–coil transition^{12–14}. Poland's algorithm including the effects of final dissociation of duplex DNA to single strands was used¹⁵. Parameter values for T_{AT} and T_{GC} , the average melting temperatures of A·T and G·C base pairs, σ , the nearest-neighbour cooperativity factor, and other thermodynamic parameters are given in Fig. 2 legend. For the short DNAs examined, the melting of internal regions and the corresponding loop entropy contribution to the theory are not significant. Proper account of dissociation of duplex to single strands¹⁶ is essential for accurate agreement with experimental results.

The total fraction of hydrogen-bonded base pairs, θ_{net} , depends on the DNA's external, as well as internal, degrees of freedom. θ_{net} may be written as $\theta_{net} = \theta_{int}\theta_{ext}$, where θ_{int} is the fraction of bonded base pairs among the duplex strands and θ_{ext} is the fraction of strands associated as a duplex with one or more intact base pairs. The equilibrium dissociation of the duplex to single strands may be represented by the reaction:



where C_2 is a duplex and C_1 is a single strand. It has been shown^{13,16} that θ_{ext} depends on Z_{int} , the internal molecular partition function of the DNA, C_0 , the total strand concentration, and β , the ratio of partition functions for the external degrees of freedom of duplex and single strands.

The mass dependence of rotational and translational partition functions of polyatomic molecules suggests that β varies with DNA length in base pairs, N , as

$$\beta = \frac{Z_{ext}(C_2)}{[Z_{ext}(C_1)]^2} = K \frac{N^{\alpha_2}}{N^{2\alpha_1}} = KN^\alpha \quad (2)$$

where $\alpha = \alpha_2 - 2\alpha_1$ and K is a constant which includes all terms independent of N ^{13,17}. α_1 and α_2 combine the N dependence of the translational degrees of freedom, $N^{3/2}$, as well as the N dependence of the rotational degrees of freedom. The latter vary according to the molecule's principal moments of inertia, for example, for a rod $N^{7/2}$, for a sphere $N^{5/2}$. Our first attempts to fit theoretical curves to experiment modelled equation (1) as a rod dissociating to two spheres. This assumption yields $\alpha = -3$ for equation (2). A constant K value was selected to provide a

Fig. 1 The 95-, 144-, 203- and 301-base pair restriction fragments containing part of the lactose operon transcription initiation region. Each fragment is fully embodied within the next largest. A partial restriction map indicates location between restriction sites. *lac z* is the region coding for β galactosidase; *lac i* is the region coding for the *lac* repressor. The starting point of transcription for the *lac* operon mRNA is designated as +1. CAP, P and Q indicate the binding sites for the CAP–cyclic AMP complex, RNA polymerase and *lac* repressor respectively. Each fragment was cloned into *EcoRI* sites^{21,22}. Purified fragments thus had four-base *EcoRI* ends. The influence of these single-stranded ends on theoretical melting curves was negligible.

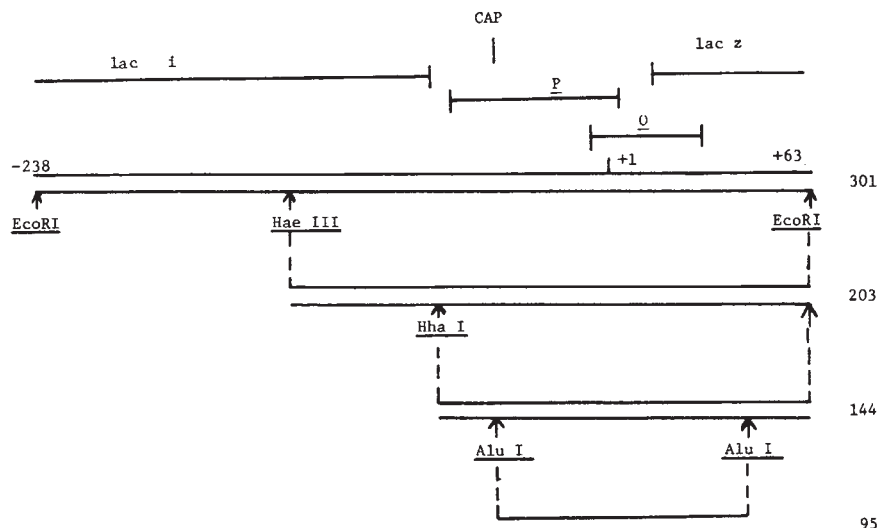
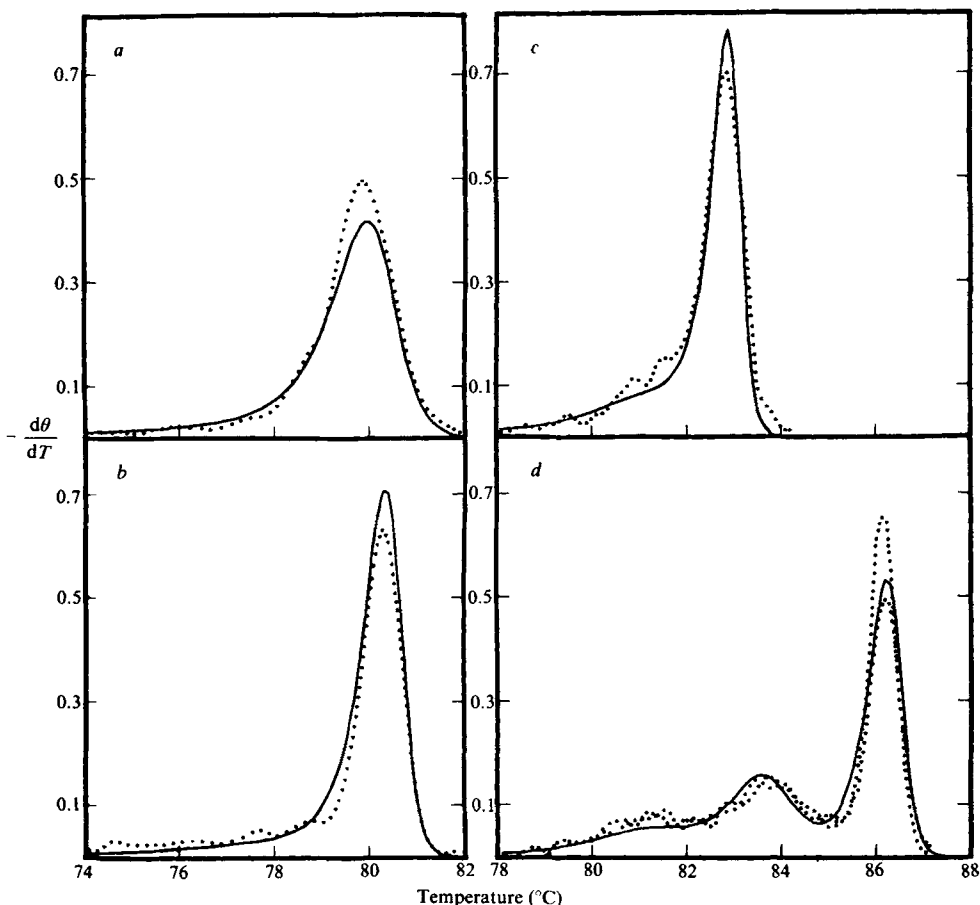


Fig. 2 Experimental differential melting curves of *lac* operon fragments (dotted lines) and theoretical curves (solid lines) are plotted. *a* Shows the 95-base pair DNA, *b*, the 144-base pair DNA, *c* the 203-base pair DNA and *d* the 301-base pair DNA. The solvent was 0.1 M NaCl, 1 mM potassium phosphate and 10 μ M EDTA. Experimental data for the 95-, 203- and 301-base pair DNAs were from Hardies *et al.*¹⁰. The 144-base pair fragment was isolated in this laboratory using previously described methods^{21,22}. Melting curves of the 144-base pair DNA were obtained in the same solvent as above. A double-beam spectrophotometer was used. A platinum resistance thermometer measured the sample temperature. The heating rate was 6 °C h⁻¹ and absorbance readings at 260 nm were made at approximately 0.05 °C intervals. Data were collected on paper tape and then processed with a time-sharing computer facility. Detailed procedures will be described elsewhere (D.K.H. and R.M.W., unpublished). The data analysis is similar to that used by Vizard *et al.*². The T_m values of the 95-, 203- and 301-base pair DNAs were reported in ref. 10. Reproducibility of these T_m s was within 1 °C. Duplicate experiments of the 144-base pair fragments gave a T_m of 80.9 $\pm$ 0.2 °C. *d* Shows the internal reproducibility of two melting curves of the 301-base pair fragment. The major peaks of the two dotted experimental curves have been aligned for comparison at the average T_m . Experimental error for the other three fragments was no greater than that shown in *d*. Calculated curves were shifted slightly in temperature (0.2–0.6 °C) to match the average T_m s of the major peaks. The small oscillatory peaks in the early part of the experimental transitions are probably due to the cubic fit smoothing of the raw absorbance data. We do not regard them as real intensity peaks. Melting curves of the 95- and 301-base pair fragments were also obtained at 282 nm. The 95-base pair curve was within the experimental error of melting transitions measured at 260 nm. The only significant difference between the 301-base pair curve at 282 nm and *d* was 20% decrease in the amplitude of the minor peak. Theoretical parameters used were $T_{AT} = 64.3$ °C, $T_{GC} = 107.2$ °C, $\sigma = 4.5 \times 10^{-5}$, $H_{AT} = -8.5$ kcal mol⁻¹ and $H_{GC} = -9.4$ kcal mol⁻¹. The loop entropy term was that used by Wartell²³. As expected, varying the loop entropy constant ($1.0 < k < 1.9$) did not alter the results. The dissociation parameter, β , is given in the text and Fig. 3 legend.



good fit to the melting curve of the 301-base pair DNA and β values for the other DNA fragments were determined from equation (2). This procedure provided very good agreement with the experimental T_m values and overall transition shape. However, the theoretical curves consistently predicted transition widths slightly broader than those observed experimentally. This discrepancy was removed by altering the simplified model to include dependence of the external partition functions on θ_{int} . The moments of inertia for various duplex configurations clearly depend on the amounts of base-paired and single-stranded regions. Figure 3 illustrates DNA configurations at different stages of melting. We assume that within the helix-coil transition region, DNA melting resembles a rod dissociating to two coiled spheres for the initial stages of melting and a sphere dissociating to two coiled spheres at the upper end of the transition. Thus α varies as $\alpha = a + b(1 - \theta_{int})$, where the constants a and b are chosen such that α decreases from -3 to -4 through the transition. This change removed the transition-width discrepancy.

Theoretical calculations can determine θ_i , the probability that the i th base pair of the sequence is intact at a given temperature^{14,15}. Melting profiles—plots of $(1 - \theta_i)$ against i as a function of temperature—indicate the temperatures at which specific regions within the DNA sequence unwind. Note that θ_i depends only on a DNA's internal degrees of freedom, that is,

$$\left(\sum_{i=1}^N \theta_i \right) / N = \theta_{int}.$$

Figure 4 shows melting profiles of the 301-base pair fragment between 80 and 90 °C. Similar profiles have been obtained for the 95-, 144-, and 203-base pair fragments (not shown). The profiles indicate that denaturation initiates from the ends with regions of 60–80 base pairs melting over a 1–2 °C interval. These regions can sometimes be associated with peaks on experimental

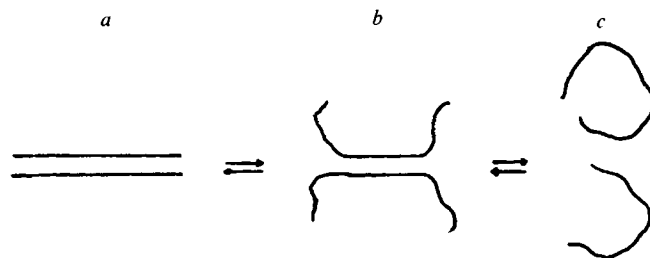


Fig. 3 Simple model for short DNA helix-coil dissociation. Three configurations are labelled A (helix rod), B (helix rod-sphere) and C (two coiled spheres). β , the ratio of partition functions for the external degrees of freedom, depends on fragment length N , and the fraction of bonded base pairs among duplexed strands, θ_{int} . $\beta = KN^\alpha$, where $\alpha = a + b(1 - \theta_{int})$. Constants K , a and b were 5×10^3 , -2.8 and -3.2 respectively. a and b were chosen such that when combined with θ_{int} , α varied from -3 to -4 through the transition. In the early stages of the transition, $\theta_{int} \approx 0.9$, $\alpha \approx -3$ and dissociation is best described as $A \rightarrow C$. Base-pair unwinding with increasing temperature changes the configuration of the partially duplexed DNAs. Duplex dissociation resembles $B \rightarrow C$ as the temperature increases. For the last portion of the transition, $\theta_{int} \approx 0.6$ and $\alpha \approx -4$.

curves. Figure 4 shows the right-most 52 base pairs melting by 82 °C. This region corresponds to the slight increase from 80–82 °C in Fig. 2d. The small peak centred about 83.6 °C in Fig. 2d is associated with the melting of an 88-base pair block adjacent to the previously unwound region. These results are similar to those deduced by Hardies *et al.*¹⁰ from analysis of the experimental curve and base-composition distribution of the 301-base pair fragment. One must be careful not to overinterpret melting profiles such as those in Fig. 4. As θ_i values do not consider strand dissociation, a meaningful comparison of melting profiles with differential melting curves is only possible when most strands are in the duplex state. Figure 4 shows an 80-base pair region largely intact at 88 °C, where the net fraction of intact base pairs, θ_{net} , is close to zero (Fig. 2d). At this temperature $\theta_{\text{int}} \approx 0.3$ but $\theta_{\text{ext}} \approx 10^{-5}$. Strand dissociation has reduced duplex DNAs to an insignificant fraction of total strands. Figure 4 would be valid throughout the transition if one could cross-link the complementary strands to prevent strand dissociation. Melting profiles of the 95-, 144- and 203-base pair fragments also show that melting initiates from the ends.

The agreement between theory and experiment indicates that the theoretical model is accurate for the conditions examined. Further studies on other short DNA restriction fragments also show good theory–experiment agreement (W. Hillen, T. C. Goodman, R. D. Wells, A.S.B. and R.M.W., unpublished results). It remains uncertain why exact calculations do not agree well with experimental curves of longer DNAs. The theory provides a method for examining the influence of base pair sequence on DNA melting of short fragments. Additional calculations show that single base pair changes in certain locations

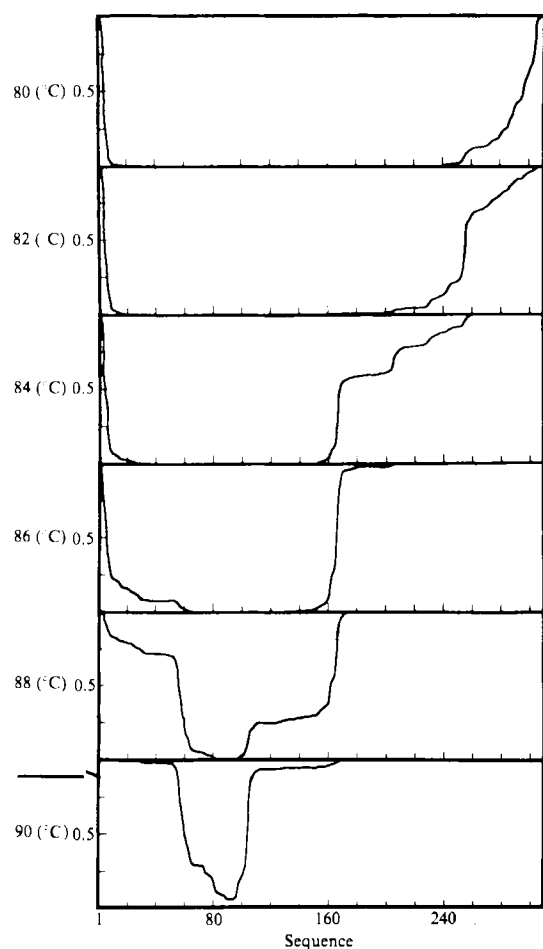


Fig. 4 Probability profile for the 301-base pair fragment. Probability of a base pair broken ($1 - \theta_i$) is plotted on the y ordinate against base pair (i) of the 301-base pair DNA sequence as a function of temperature.

in the 203-base pair DNA can alter the overall melting temperature by 0.6 °C. This type of information may be relevant to understanding the interaction of RNA polymerase with promoter sites. Several results indicate that the thermal stability of promoters and their surrounding regions influence RNA polymerase–promoter interaction^{18–20}.

We thank S. C. Hardies, W. Hillen, T. C. Goodman and R. D. Wells for providing us with their manuscript before publication, and for helpful discussions and supplementary information. This work was supported by the NSF grant PCM 76-04565, Biomedical Research Support grant 5-S07-RR07024, and a grant of computing time from the Office of Computing Services at the Georgia Institute of Technology. R.M.W. holds a Research Career Development Award of the NIH.

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Detection of the effects of asymmetric assortative mating

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Assortative mating is clearly important in determining the genetic structure of a population but studies of assortative mating in man have been restricted mainly to the examination of correlations between spouses for single variables. It has been assumed that assortation is based directly on the measured phenotype or on components of the phenotype whose determination is similar in both sexes. Recently, such assumptions have been questioned (see, for example, refs 1–3) because males and females may regard different aspects of the phenotype as important for mate selection. The term ‘asymmetric assortative mating’ has been used to describe such situations. It has also been suggested that the study of the spouses and offspring of twins^{1,2} may permit resolution of such components of the mating system. *Ad hoc* models have been proposed (for example, ref. 1) to specify the contribution of asymmetric assortment to the correlations between relatives, but these have not been internally consistent or sufficiently parsimonious. We show here how a simple model based on biological considerations offers a foundation for a consistent and economical theory of asymmetric mate selection which may be used for the analysis of patterns of familial similarity.

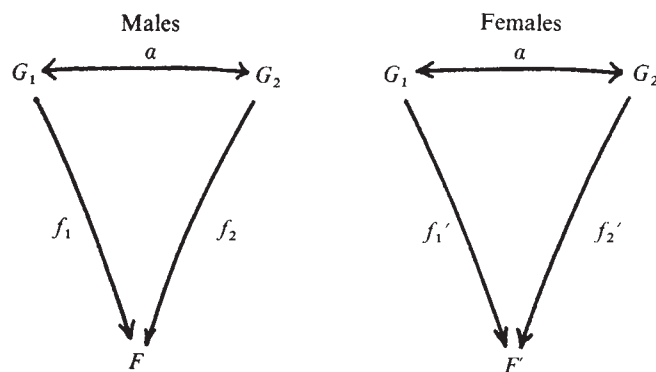


Fig. 1 The contributions of two genetic systems to variation in male and female fitness. See text for definition of symbols.

The model assumes that assortative mating is based on a composite aspect of the phenotype, 'fitness', and is necessarily symmetric at this level. Asymmetry in mate selection arises because the sexes differ in the contribution of particular genetic effects to this composite phenotype, thus the determination of fitness is partly sex-limited. The principles are illustrated by a simple special case in which there is no environmental variation in fitness. The model can be extended to incorporate such additional subtleties as maternal effects and cultural transmission without altering its essential features.

For simplicity we postulate that fitness in males, F , and in females, F' , is determined by two genetic systems, G_1 and G_2 , which are correlated within individuals simply through the genetic consequences of the mating system. The contributions of G_1 and G_2 to variation in F and F' are represented by the standardized regression (path) coefficients f_1 and f_2 for males and f_1' and f_2' in females. The correlation between G_1 and G_2 is denoted by α . The possibility of asymmetry is represented by the fact that the paths in males and females, f_1 and f_1' , f_2 and f_2' are not necessarily the same. Figure 1 represents the model in diagrammatic form.

It is assumed that assortation is based primarily on the phenotypic correlation (which is symmetric by definition) between F and F' . This is the marital correlation, μ , for fitness. The critical determinant of the distribution of genetic

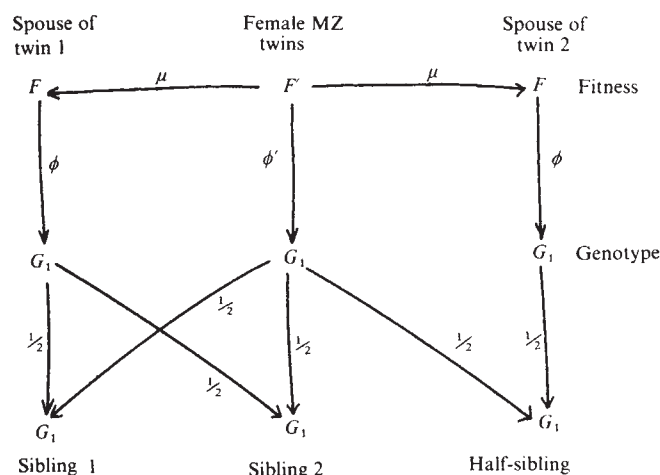


Fig. 2 The contribution of assortative mating to the genetic similarity of the relatives of female identical twins.

Table 1 The genetic correlation between MZ twins and their relatives in the presence of asymmetric assortative mating for fitness

Genetic correlation	Sex of twins	
	Male	Female
Twin	1	1
Twin-spouse	$\phi\phi'\mu$	$\phi\phi'\mu$
Spouse-spouse	$\phi'^2\mu^2$	$\phi^2\mu^2$
Sibling	$\frac{1}{2}(1+\phi\phi'\mu)$	$\frac{1}{2}(1+\phi\phi'\mu)$
Half-sibling	$\frac{1}{4}(1+2\phi\phi'\mu+\phi'^2\mu^2)$	$\frac{1}{4}(1+2\phi\phi'\mu+\phi^2\mu^2)$
Parent-offspring	$\frac{1}{2}(1+\phi\phi'\mu)$	$\frac{1}{2}(1+\phi\phi'\mu)$
Spouse-nephew/niece	$\frac{1}{2}(\phi'^2\mu^2+\phi\phi'\mu)$	$\frac{1}{2}(\phi^2\mu^2+\phi\phi'\mu)$

See text for definition of parameters.

differences in the next generation is the genetic correlation between spouses. We consider that between the deviations in G_1 for males and females. The algebra is equivalent for any other component of F and F' , whose effects may be measured directly or indirectly. Given α , μ , f_1 , f_2 , f_1' and f_2' , the correlation between the G_1 s of spouses is $\mu\phi\phi'$ where $\phi = f_1 + f_2\alpha$, and $\phi' = f_1' + f_2'\alpha$. As both ϕ and ϕ' are involved in this correlation, they will not be resolved by examining the phenotypic correlation between spouses for a single variable. Such resolution is possible, however, if we consider the spouses of relatives. Following Nance¹ and Eaves², we consider the spouses of identical (MZ) male and female twins. To explore the consequences of asymmetry for the next generation we also consider the genetic correlations between siblings and the biological half-siblings obtained by mating MZ twins to separate spouses. We consider only the effects of G_1 , as shown in the model in Fig. 2. The coefficients of $\frac{1}{2}$ on the paths between parental and offspring genotypic deviations reflect an underlying assumption of genetic additivity. The diagram assumes that the twins are female. The figure for male twins would be similar except that ϕ would be replaced by ϕ' and vice versa.

The expected genetic correlations between relatives are given in Table 1. These can be obtained by applying the rules of path analysis to the model in Fig. 2. The sex of the twins does not affect the sibling and parent-offspring correlations differently. The maternal and paternal half-sibling genetic correlations differ by $\mu^2(\phi - \phi')$ and that between the spouses of female and male twins by $\mu^2(\phi^2 - \phi'^2)$. As Nance¹ suggested, the effects of asymmetry may indeed simulate those of classical maternal effects in half-sibship data by making maternal half-siblings more alike than paternal half-siblings if $\phi > \phi'$. It is the comparison of the spouses of twins which may have a critical role in the resolution of direct maternal effects from those created or simulated by asymmetric mate selection. Environmental components in the determination of F and F' change the definition of ϕ and ϕ' without substantially altering these conclusions. Clearly, resolution of genetic and environmental determinants of mating preference requires data on the spouses of relatives in addition to MZ twins², but the model we propose may form the basis for the discussion of more complex systems of mating than those which are frequently considered by students of human differences.

A. C. H. holds a MRC studentship. The work is part of a programme supported by the MRC and of a project supported by the SRC.

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BOOK REVIEWS

Anti-realist philosophy of science

Mary Hesse

PHILOSOPHERS of science have recently been much exercised about the question "Do scientific theories describe the real world?". This is a question that arises from two sorts of problem. First, it has become a question within quantum physics, and has led to disputed interpretations such as those between the so-called Copenhagen school and the "hidden variables" theorists. Second, it has become a matter of particular concern to philosophers since the radical analyses of Kuhn and Feyerabend in the 1960s, in which it was suggested that the contents of particular scientific theories are culturally relative pictures of the world, not attempts at true objective explanation. The villagers of Salem believed in witches, modern scientists believe in atoms; both are unobservable, so why should the latter be considered to be nearer the truth? Even to raise such a question constituted an attack upon the rationality and progressiveness of science, and sent philosophers scurrying back to the defence of realism.

Van Fraassen's book marks a significant new stage in this debate. In it he defends an anti-realist position, but without falling into the extreme absurdities of relativism. He draws the lines of the debate in the following way. What he calls "minimal realism" holds that "science aims to give us, in its theories, a literally true story of what the world is like; and acceptance of a scientific theory involves the belief that it is true" (p. 8). What he calls "constructive empiricism", on the other hand, holds that "science aims to give us theories which are empirically adequate; and acceptance of a theory involves as belief only that it is empirically adequate" (p. 12). His arguments against the former view and for the latter are detailed and cogent, and range over the problematic relation of theory and observation, the nature of scientific explanation and scientific methodology, and the proper interpretation of "probability" in statistical science. I will pick out a few of the salient and more controversial points of the argument.

First, as van Fraassen construes the debate, it is about the aims of science, not about the nature of theories as such. He does not claim that theories are "nothing but" computing devices for processing and emitting predictions, nor that they are metaphorical fictions which speak only

The Scientific Image. By Bas C. van Fraassen. Pp.235. (Clarendon/Oxford University Press: 1980.) Hbk £15, \$45; pbk £6.50, \$18.95.

and misleadingly about observations. For van Fraassen, theories consist of meaningful propositions having truth value. They are given meaning in the same way that any descriptive language is given meaning (whatever that is). Van Fraassen does not consider it the job of philosophy of science to debate general theories of meaning, and here he reveals a belief that science and the philosophy of science do not fundamentally upset the *status quo* with regard to received theories of language, meaning and truth. Thus he does not follow those radical philosophers of science who consider that scientific concepts may so modify our commonsense picture of the world as to require changes also in the way we conceive natural language. van Fraassen does not believe that philosophy of science has implications for "deep" philosophical problems, or that philosophy of science is properly about "language" at all — it is about the world, just as science itself is about the world. Such conservative assumptions are controversial, but van Fraassen is certainly correct in seeing that if they were to be questioned, we would be led into issues far beyond the range of this book.

Theories, then, have truth value, but it is not the aim of science to discover true theories. Though it is not argued quite explicitly in the book, this rejection of theoretical "explanation" as the chief aim of science seems to be based on the belief that we can never know that we have arrived at exactly true theories. Most theories have turned or will turn out to be false as more evidence comes in, and even where they have not yet been refuted, there are always conceivable incompatible alternatives which are also empirically adequate. Thus the aim of discovery of true theories is one that we cannot ever know that we have attained, and it should be replaced by the realizable and often realized aim of empirical adequacy. To the currently fashionable argument that theories must be true of the real world because they are so successful in application, van Fraassen simply replies that they are successful because they incorporate accounts of real empirical

regularities; it is these that make them successful, and only those theories that incorporate them survive. But we have no warrant to claim truth for what goes beyond empirical regularities, even though all interesting theories do undoubtedly go beyond them.

Other common arguments for and against realism have started from the so-called "theory-ladenness" of observation. On behalf of realism it is argued that since our theoretical view of the world reaches right down into the way we describe observations ("tracks of *electrons*", "*glacial moraines*") and into the expectations we form regarding possible future observations, therefore we are bound to presuppose that at least some of our background theories are true. On the other hand, the anti-realist replies that this all-pervasive character of theory simply shows that our theoretical world-views are cultural products, which can be seen in historical and social perspective to change from culture to culture, and are therefore culturally relative and not realist. Van Fraassen makes short work of all this argumentation. Our language, both theoretical and observational, is of course the language of *our* culture group. But this does not make observational *information* either theory- or culture-relative — only its *expression* as embodied in the current scientific language. Theories are not linguistic entities; they should rather be seen as families of models of possible worlds, whose empirical sub-structures represent phenomena.

Talk of models commits van Fraassen to a metatheoretical view of science in the sense that he presupposes that theories are ideally axiomatized, and that their empirical content is exhausted by the empirical interpretations of entailments of the axioms. This view results in what I would regard as a major limitation of the book, namely van Fraassen's treatment of scientific methodology. He sets out to show that accepted methodological principles such as simplicity, explanatory power, the unity of science and so on are explicable in terms of an anti-realist view. In some cases he succeeds, but in others he has to be content to regard the maxims of theory-choice as "merely pragmatic", that is to say not relevant to questions of truth or the empirical adequacy of predictions. In terms of his position he cannot answer

questions such as: why should predictions from simpler theories be generally more reliable?; why should analogical arguments from theory to theory work as well as they do? I believe van Fraassen's comparatively cavalier dismissal of such problems of epistemology, induction and confirmation can be traced to his exclusively deductive (and sometimes statistical) construal of the formal character of theories. But theories are not treated in scientific practice as axiomatized entities; they have much fuzzier edges than that, and reliably support inferences that are not just deductive or formally statistical.

Such limitations apart, however, this book for the first time makes a clear statement of what is involved in scientific realism, and it will become the classic

current argument against that philosophical position. It successfully debunks recent exaggerated attention to the concept of "explanation", and replaces it with a healthy concern with truth and empirical adequacy that does not give any hostages to relativism. Tactically, its relative conservatism about the general philosophical implications of philosophy of science will make it all the more influential among realist philosophers. Scientists, however, may well be ready for more radical investigation of the effects of changing scientific theories on our commonsense presuppositions, and particularly on our views of logic, natural language and scientific inference.

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of their predicament in a new way. There is no doubt but that this is a benefit. National revival is unilaterally possible (such as occurred in France in 1958) but it is unlikely to occur save in the climate of international prosperity.

W. H. McNeill is no newcomer to the grand approach. His *Rise of the West* (Chicago University Press, 1963) is rightly regarded as one of the best, probably the best, of the conventional outline general histories. His *Plagues and Peoples* (Blackwell, 1977) was an extremely interesting introduction to the role of disease in history. *The Human Condition* is more modest, being merely two (long) lectures given at Clark University. Or at first sight modest, because, after all, there is a sense in which any book with this title which seeks adequately to cope with such a subject-matter in 75 pages is magnificently ambitious.

The themes of Professor McNeill's essay (for such it is) derive from his earlier study of disease. The main concept is what he calls "parasitism", divided in its challenge to human beings into "macroparasites" and "microparasites". Microparasitism refers to the "metabolic activities of minute organisms" which compete with human beings for food: by getting there first, microparasites can forestall human efforts to capture energy from food. Macroparasitism is any "exploitative relations among groups and classes of human beings", such as when one man or group of men — armed raiders, tax collectors, rent collectors — seize goods or compel services from other groups.

This rather forbidding frame gives Professor McNeill a justification for what turns out to be an extremely lively and well-written short world history, with the use of little jargon other than that I have already quoted. The frame enables Professor McNeill to make some interesting and novel points: for example, he attributes the advance of our remotest ancestors to the "apex of the food chain" to the acquisition of language and to the superior co-ordination of human behaviour that language allowed. The next landmark was the use of clothes to "maintain a tropical microenvironment" next to the almost hairless skin we all inherit. A bearskin on the back enabled us to live in a cold climate. Agriculture, though a definite benefit, Professor McNeill somewhat grudgingly confirms, brought microparasites as well as harvests. Irrigation brought mosquitoes. The "first recorded conqueror" Sargon of Akkad (c.2250 BC) was the first macroparasite on an imperial scale. Civil servants were the consequence — a problem with which we are still wrestling. In the sixteenth century, a fundamental change occurred, worthy of greater attention than Professor McNeill gives it — businessmen succeeded in establishing real autonomy from their political superiors, with even the mightiest European rulers depending on traders and bankers to

Archaeological jargon explained

A. F. Harding

A Dictionary of Terms and Techniques in Archaeology. By Sara Champion. Pp.144. (Phaidon/Facts on File: 1980.) Hbk £6.95, \$15.95; pbk £4.95.

THE Introduction to this little volume tells us that the book "is not a general dictionary of archaeology: names of cultures and artefacts have been deliberately omitted". Instead, it "has been compiled with the aim of introducing to non-professional archaeologists the terms and techniques used in modern archaeology". The author disclaims personal expertise in most of the techniques described, and has generally provided brief (typically 30 to 300 word) entries with a reference to a standard work on the subject.

Despite its limited brief, professionals and students, as well as interested

amateurs, will find much of practical use in this book, especially for checking up on those jargon terms one so frequently hears but secretly wonders about. The *Dictionary* contains only about 330 entries, many of them illustrated, so the scope is necessarily selective: most space is devoted to modern archaeological theory, scientific techniques in archaeology, archaeological techniques *per se*, terms from related disciplines and a certain number of technological terms — especially those relating to potting and metalworking. Stone and stone-working, to take a single example, are barely touched upon.

Each reader will undoubtedly find his own lacunae, and it is a pity that the author was not able to roam more freely. Yet the book is well-produced and within its self-imposed limits can be recommended.

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The biology of history

Hugh Thomas

The Human Condition. By W. H. McNeill. Pp.75. (Princeton University Press: 1980.) £4.75, \$11.25.

UNIVERSAL histories are becoming alarmingly fashionable. This is understandable for three reasons. First, although we are a long way still from anything like a world state we are quite close to an interdependent world society. The slightest hiccup in southern Asia affects northern Europe. Oil, especially, is the great

internationalizing force, causing all responsible people to remember statistics of production in countries whose very names were unknown to them ten years ago. Second, the concentration of such enormous power in the hands of two super-powers is, also, a development which has never occurred before in human history: until 1945, power was always far more widely dispersed. The third explanation is that the apparent long-term shortages in raw materials have persuaded human beings to reflect seriously over the springs

organize an efficient army. They had to solicit loans from private persons who were usually domiciled outside the ruler's own jurisdiction and so were safe from confiscatory taxation. This really began the modern age — despite Professor McNeill's encouragement to us to look at the Sung dynasty in China AD 960–1279 in this light. (Incidentally, there is a certain discrepancy between Professor McNeill's comments on mediaeval China — p.48 — and his comparison with Renaissance Europe — p.62; he seems really to stand by the latter interpretation where he says that private

businessmen in China never won autonomy from their superiors nor accumulated capital on the scale that European entrepreneurs did.)

The last pages of Professor McNeill's book are provocative and interesting and end on a sensible note: "better hindsight deepens insight". Foresight also will be assisted by all sensible enough to get hold of this excellent little book.

Hugh Thomas was formerly Professor of History at the University of Reading.

Alternative energy: the biological option

C.R.W. Spedding

Biochemical and Photosynthetic Aspects of Energy Production. Edited by Anthony San Pietro. Pp.231. (Academic: 1980.) £15.80, \$28.

AS THE editor of this book points out in his preface, photosynthesis is the only method of solar energy conversion currently practised on a large scale. Increasing dependence on fossil fuels is now an extremely vulnerable condition, and this volume explores the potential of biological processes that may serve in the future to provide alternative energy resources.

Eleven contributors discuss, in considerable detail, various aspects of this enormous subject. D.O. Hall provides an introductory overview and reviews the development of photobiological conversion systems and their long-term implications from the point of view of both energy and food. He stresses the need to

consider all our energy options and not repeat the mistake of putting all our eggs in one basket.

Four chapters deal with different forms of energy from biomass production systems based on seaweed aquaculture, algal-bacterial systems, anaerobic fermentation of residues and biomass, and energy crops. All of these need further exploration; some are not yet established as technically feasible and all depend for their relevance on relative costs. This presents a major problem: how to estimate costs and prices for the future for technologies that are currently at a stage equivalent to the first motor car or aeroplane. The value and role of energy analysis is considered, but this cannot be a complete answer without regard to the fact that one joule of energy is not necessarily of the same value as another.

Several contributors emphasize the

importance of non-energy products in our assessments. In some cases, such as glycerol production from the alga *Dunaliella* spp., the output is predominantly of one substance, but the economics of most processes will depend upon a use for all of the fractions produced.

Other issues considered are the atmospheric consequences of various developments and the biological fixation of nitrogen. The latter is of key importance to greater and energetically more efficient production of biomass.

The book is well produced, has a useful index and comprehensive lists of references, and represents a valuable summary of the state of an important subject.

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Polyamines reviewed

Anthony E. Pegg
Hannu Pösö

Polyamines in Biomedical Research. Edited by Joseph M. Gaugas. Pp.474. (Wiley-Interscience: 1980.) £30, \$90.

KNOWLEDGE of the importance of polyamines in cellular physiology has been greatly extended in recent years by studies of the control of polyamine biosynthesis, the development of drugs interfering with polyamine accumulation and improved technology for measurement of these compounds and derivatives. Many reviews on these subjects have appeared during the past few years and this book is the latest in the series. It contains a number of outstanding chapters and, though dated, will be of considerable interest to those working in the field.

The book is a collection of 27 review chapters by different authors. About one-third of these are concerned with the control of polyamine synthesis and the role of polyamines in cell growth. There are naturally substantial overlaps in which different authors describe results from the same publications but all of these articles are by experts in the field and are of interest. Chapters by Stevens and Stevens and by Mamont, Bey and Koch-Weser give comprehensive and well-written coverage of inhibitors of decarboxylases involved in polyamine biosynthesis. This provides excellent reference material, as does the contribution on regulation of ornithine

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REASONS

Erratum: see *Nature* 288, 630. The title of the book written by Sandra Raphael and Wilfrid Blunt is *The Illustrated Herbal* (Frances Lincoln/Thames & Hudson, £12.50, \$25).

Still small — photobiological conversion systems. An experimental tubular loop reactor which converts artificial solar energy into biomass.

decarboxylase by McCann. The possible importance of polyamines and ornithine decarboxylase in skin tumour promotion and as markers for compounds with anti-promoting actions are well described by O'Brien and by Verma and Boutwell who were pioneers in the field. There is also a first-rate and — at the time of writing — comprehensive evaluation of methods for polyamine measurement by Seiler who is responsible for much of the methodology now in routine use. Isolation and analysis of novel polyamines found in thermophiles are covered in a chapter by Zappia and colleagues. The remainder of the book contains a heterogeneous collection of contributions on carcinogenesis, polyamine degradation, their putative roles in macrophage function, relationship to chaperones, suppression of lymphocyte function, excretion and conjugation, and value as tumour markers. These articles are often highly speculative and, while thought provoking, are much less rigorous.

Several important contributors to polyamine research are missing from this volume (for example it is unfortunate that there is no chapter from H. and C.W. Tabor on their polyamine-deficient mutants), and the two-year delay between writing and publication has meant there are a number of significant omissions in content. However, as a source book for scholars of a wide range of interests, it should prove valuable.

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Air of learning

Peter S. Liss

Air in Danger: Ecological Perspectives of the Atmosphere. By G. Breuer. Pp.189. (Cambridge University Press: 1980.) Hbk £10, \$24.95; pbk £3.50, \$7.95.

IMAGINE you are a scientist or scientific journalist invited to attend a research meeting on a topic which is not your speciality. You enjoy the meeting and become sufficiently interested in the topic to follow it up by reading, and visiting and writing to experts in the field. Then you decide to write up not only what you have learnt but also the various stages in the learning process, and persuade major publishers to issue the result in both German and English.

Although no biographical details of the author of *Air in Danger* are given (and, for once, I felt their absence to be a real loss), this would appear to be the way in which this book came into being. Georg Breuer attended, as an observer, the Dahlem Conference, "Global Chemical Cycles and Their Alterations by Man", held in Berlin

in November, 1976. This spurred him to find out more about a major topic of the meeting, the way in which the major atmospheric gases — nitrogen, oxygen and carbon dioxide — are cycled in the environment. The chapters of the book chronicle the author's education as to how normal biological processes transfer these elements between the various environmental reservoirs and also how humans are influencing this cycling. Quite properly, considerable attention is given to the photosynthesis/respiration-decomposition reactions since these truly dominate the environmental chemistry of the gases considered, as well as that of many other substances.

Inevitably, this way of setting about writing a book has its disadvantages. Someone who is self-taught spends considerable time exploring what turn out to be blind alleys (for example, several pages in the first chapter deal with what is well known to be the "non-problem" of whether fossil fuel combustion can significantly deplete atmospheric oxygen) and also tends to over-detailed explanations.

On the plus side the approach adopted

has the freshness inherent in knowledge newly acquired. What is not clear is what type of reader will want to share in the author's learning experience, since each of us learn in an individualistic and idiosyncratic manner. The book is certainly timely in that it presents in considerable detail much of the background behind the current debate over Lovelock's Gaia hypothesis, recently expounded in his book *Gaia: A New Look at Life on Earth* (Oxford University Press, 1979 — see *Nature* **282**, 154 for review).

The translation (by Peter Fabian) from the German original reads very well, but the English edition should never have been titled as it is. The main title gives the impression of a gloom and doom, pollution-orientated book which it certainly isn't. In fact, the author is to be commended for treading a very sensible line between natural and anthropogenic effects and the book's main title does his care a considerable injustice.

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Stimulating energy transfer

A.J. McCaffery

Introduction to Molecular Energy Transfer. By James T. Yardley. Pp.308. (Academic: 1980.) \$34, £19.20.

THE microscopic processes of energy transfer — the molecular-level events in the gas, liquid and solid phases of matter — have been the subject of intense research interest for a number of years, and a book which attempts to draw together the wide-ranging strands of this field is most welcome. The topics covered in this volume reflect both the author's own research interests and the major thrusts of recent research, and thus the bulk of the book deals with vibrational energy transfer. Some of the modern experimental methods are reviewed in detail, as, more briefly, are the classical methods of ultrasonic dispersion and shock tube techniques. Gas phase vibration-to-translation and vibration-to-vibration transfer both receive detailed treatment and there is a short chapter on vibrational relaxation in liquids and inert materials. Included as a basis for the treatment of vibrational processes is a comprehensive theoretical development of the internal vibrational modes of molecules.

The remainder of the book is taken up with discussion of a diverse and interesting collection of topics. These range from radiationless electronic transfer in molecules, to the less known phenomena of collision-induced fine structure transitions in atoms and quenching of atomic

excitation by curve crossing.

One interesting feature is a derivation of the Born approximation as early on as p. 8, and its subsequent usage runs like a thread through the wide range of fields covered in the later chapters, providing a useful physical insight into the individual transfer processes.

The difficulties of producing a completely up-to-date text are apparent, though references to work as late as 1978 are relatively frequent in some sections. Perhaps a more substantial criticism is that the book's purpose is not entirely clear. It is a mixture of pedagogy, in that it covers such basics as group theory, the hydrogen atom, LCAO-MO model and determinantal basis functions, and review. The coverage of rotational energy transfer is slightly disappointing by comparison to the treatment of its vibrational counterpart, as is the absence of discussion of scattering theoretic, semi-classical and classical trajectory studies of energy transfer.

Despite these small criticisms, Yardley's book is a stimulating and readable account of an important research topic with a number of interesting and novel features in its development. It should prove popular as a basic graduate text for North American universities and as a valuable source of methods and theory to research workers.

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